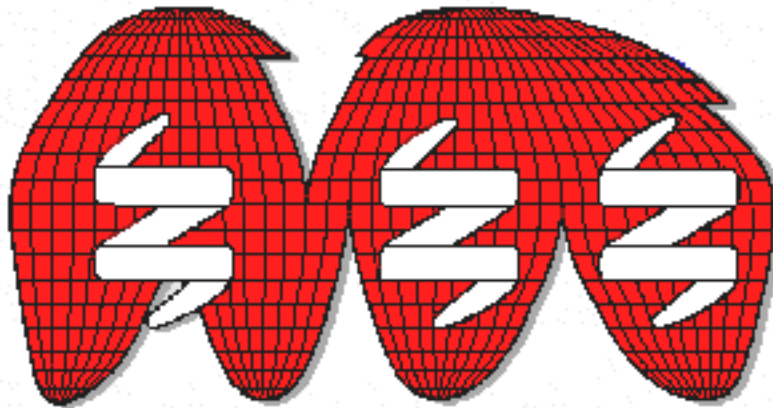


*XIth International Conference on Trichinellosis*



*ICT - 11*

August 8-12, 2004

Douglas F. Manchester Conference Center  
University of San Diego  
San Diego, California

# Table of Contents

	Page(s)
Title Page .....	1
Table of Contents .....	2
ICT-11 Organizing Committees .....	3
Financial Support .....	4
Acknowledgements .....	5
Program .....	6
Social Events .....	18
Abstracts .....	19
Participant List .....	88
Author Index .....	91
ICT Membership List .....	93
Notes .....	97

# ICT-11 Organization

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## **Acknowledgements**

The Organizing Committee wishes to express our sincere gratitude to the following people:

**Anita Hesser** for the many contributions she has made in support of ICT-11, including receiving and processing abstracts, working with authors and session chairs to organize the program, preparing the program book and on-site meeting support.

**Ann Marie Lambillotte** and **Alison Knoth** for their valuable assistance in local planning and arrangements in the San Diego area.

# ICT - 11

*XIth International Conference on Trichinellosis*

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## **Sunday August 8, 2004**

3:00-7:00 pm	<b>Registration</b>
5:00 pm	ICT Executive Committee Meeting
6:00 pm	<b>Opening ceremony</b>
7:00 pm	<b>Reception</b>

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## **Monday August 9, 2004**

8:30-9:15 am	<b>Keynote Address:</b> <u>Brian Evans</u> , Canadian Chief Veterinary Officer, CFIA - "Maintaining public confidence in the face of threats to animal health, public health and food security"
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### **Theme - *Trichinella*, the nematode**

**Session I - *Trichinella* speciation.** K. D. Murrell and W. Cabaj (Chairs)

9:15-10:00 am	<b>Presidential address:</b> <u>E. Pozio</u> - "The broad spectrum of <i>Trichinella</i> hosts: from cold- to warm-blooded animals"
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### **10:00-10:45 am Poster Presentations**

- #3 Amplified fragment length polymorphism (AFLP) shows a high resolution power in detecting genetic variation in *Trichinella nativa*. T. Mikkonen, J. Koort, J. Björkroth, A. Sukura
- #5 Freeze tolerance of nine *Trichinella* genotypes in muscle tissue of experimentally infected pigs, horses, wild boars, mice, cats, and foxes. C.M.O. Kapel, P. Webster, A. Malakauskas, Z. Hurnikova, H.R. Gamble
- #6 Muscle distribution of sylvatic and domestic *Trichinella* larvae in production animals and wildlife. C.M.O. Kapel, P. Webster, H.R. Gamble
- #9 Identification of *Trichinella* isolates by means of 5S ribosomal DNA intergenic spacer region amplification and sequencing. A. De Bruyne, H. Yera, F. Le Guerhier, P. Boireau, J. Dupouy-Camet

- #113 Serological evidence of trichinellosis in local pigs of Nepal. D.D. Joshi, L.N. Moller, M. Maharjan, C.M.O. Kapel

10:45-12:00 am

**Oral Presentations**

- #8: High resolution analysis of genetic variability within *Trichinella* by non-isotopic single-strand conformation polymorphism and selective sequencing. R. B. Gasser, M. Hu, Youssef G. Abs El-Osta, D. S. Zarlenga, E. Pozio
- #2: Phylogenetic analysis of encapsulated and non-encapsulated *Trichinella* genotypes by studying the 5S rDNA tandemly repeated intergenic region. J.W.B. van der Giessen, M. Fonville, I. Briels, E. Pozio
- #4: Relationships between *Trichinella* and host species in Europe. E. Pozio, F. Serrano, P. Dubinsky, W. Cabaj, R. Blaga, I. Dida, D. Christensson, K. Noekler, G. Marucci, G. La Rosa
- #7: Comparative infectivity of three *Trichinella* species in ponies. C.M.O. Kapel, Z. Hurnikova, P. Webster, L.N. Møller, A. Malakauskas, K. Noeckler, E. Pozio, P. Boireau
- #1: A new phylogenetic hypothesis for the genus *Trichinella*. D. S. Zarlenga, B. Rosenthal, G. La Rosa, E. Hoberg, E. Pozio

12:00-1:30 pm

**Lunch**

**Session II** – Biology: Genomics/proteomics. D. Jasmer and D. Zarlenga (Chairs)

1:30-2:00 pm

M. Dautova Mitreva - “Discovery of candidate developmentally expressed genes in *Trichinella spiralis*”

2:00-3:30 pm

**Oral Presentations**

- #18 Analysis of low complexity regions of the *Trichinella spiralis* genome for the resolution of polymorphic molecular markers. G. La Rosa, G. Marucci, E. Pozio
- #21 Proteomic analysis of the excreted/secreted protein fraction of the *Trichinella spiralis* muscle larva. M.W. Robinson, D. Gare, B. Connolly
- #16 Cloning of a surprising DNase II family from *Trichinella spiralis*. M.Y. Liu, B.Q. Fu, X.P. Wu, C.M.O. Kapel, Q. Lu, C.Y. Li, Q.J. Chen, P. Boireau
- #22 Identification and characterization of a stage-specific cDNA from adult worm of *Trichinella spiralis* that encodes a caveolin-1 protein. R. Hernández-Bello, R.M. Bermúdez-Cruz, P. García-Reyna, L. Mingyuan, F. Le Guerhier, P. Boireau, G. Ortega-Pierres

#19 Cloning and expression of a glutathione-S-transferase of *Trichinella spiralis*. C. Bahuon, F. Le Guerhier, D. Le Rhun, E. Le Naour, P. Boireau

#20 Identification of potential mediators of nurse cell transformation from *T. spiralis*. D.B. Guiliano, K. Gounaris, M.E. Selkirk

3:30-4:30 pm

### Poster Presentations

#10 Genes encoding newborn larvae-specific, glutamic acid-rich proteins from both encapsulated and non-encapsulated *Trichinella* species. D. S. Zarlenga, P. Boyd, M. B. Chute, D. Hill, J.R. Lichtenfels

#11 Molecular cloning and characterization of a novel antigen from *Trichinella spiralis*. X. Zhu, J. Yang, Y. Yang, D. Li, S. Huang, L. Zhou, P. Boireau, B. Zhan, P. Hotez

#12 Expression of the 30-mer peptide from *Trichinella spiralis* 43-kDa antigen on the surface of *E. coli* BL-21 and attenuated *Salmonella typhimurium* using the autotransporter MisL. A.M. Castillo Alvarez, N. Villegas-Sepulveda, P. Ruíz-Olvera, R. Fonseca-Liñán, L. Yepez-Mulia, C. González-Bonilla, G. Ortega-Pierres

#13 Immunoscreening of an adult worm cDNA Library of *Trichinella spiralis*: Cloning of a putative serine protease family in six variant types. B.Q. Fu, M.Y. Liu, P. Boireau, Y.L. Zhang, X.P. Wu, L.H. Yuan, L.R. Li, Q.J. Chen, C.M.O. Kapel

#14 Cloning and identification of a cDNA encoding p46 kDa antigen from newborn larvae of *Trichinella spiralis*. M.Y. Liu, B.Q. Fu, P. Boireau, L.H. Yuan, X.P. Wu, Y.L. Zhang, L.R. Li, Q.J. Chen, C.M.O. Kapel

#15 Cloning and analysis of a novel cDNA encoding a putative protein with FYVE zinc finger domain of *Trichinella spiralis*. B.Q. Fu, M.Y. Liu, C.M.O. Kapel, X.P. Meng, Q. Lu, X.P. Wu, Q.J. Chen, P. Boireau

#17 Cloning of a gene encoding the cuticle collagen of *Trichinella spiralis*. B.Q. Fu, M.Y. Liu, C.M.O. Kapel, Q. Lu, X.P. Wu, C.Y. Li, Q.J. Chen, P. Boireau

#122 Two dimensional electrophoresis and mass spectrometry (MALDI TOF) for the identification of species-specific *Trichinella* antigens. M. A. Dea-Ayuela, F. Bolás-Fernández

4:30-5:30 pm

**Roundtable discussion:** D. Zarlenga, D. Jasmer, P. Boireau (Discussion leaders)

Mining the databases, genetic manipulation of *Trichinella*, *in vitro* systems



**Tuesday August 10, 2004**

**Session III – Biology: The host parasite interface. B. Connolly and Y. Takahashi (Chairs)**

- 8:30-9:00 am            D. Despommier - “The biology of *Trichinella*: what we still need to know”
- 9:00-9:30 am            K. Gounaris - “A potential role for *Trichinella spiralis* secreted proteins in modulation of host purinergic signalling”
- 9:30-10:15 am           **Poster Presentations**
- #23            Studies on vertical transmission of *Trichinella* spp. in carnivores, pigs, and rodents. P. Webster, C.M.O. Kapel
- #24            Intestinal establishment and reproduction of adult *Trichinella* spp. in mono and mixed infections in foxes (*Vulpes vulpes*). P. Webster, C. M.O. Kapel
- #25            Congenital transmission of trichinellosis in the mice. J.Cui, Z.Q.Wang, H.M.Hang
- #26            The effect of progesterone in the *Trichinella spiralis* infection. G.G. Nuñez, T. Gentile, S.N. Costantino, S.M. Venturiello
- #29            Clinical, hematological, biochemical and economic impacts of *Trichinella spiralis* infection in pigs. M. Ribicich, H.R. Gamble, A. Rosa, A. Marquez, G. Mira, N. Cardillo, M.L Cattaneo, A. Franco
- #30            Rattus norvegicus albino as a highly susceptible laboratory animal for maintenance of mongoose derived *Trichinella* larvae in Iran. G.Mowlavi, J.Massoud, S. Soleymani Mohammadi, K.Ashrafi, S.Naddaf, I.Mobedi
- #31            Evaluation of the infectivity of *Trichinella papuae* and *Trichinella zimbabwensis* for equatorial freshwater fishes. E. Pozio, G. La Rosa
- #32            Infectivity of *Trichinella* spp. in red foxes. C.M.O Kapel, P. Webster, A. Malakauskas
- #34            Increased expression of a new antioxidant enzyme in the nurse cell during *Trichinella britovi* infection as revealed by "in situ" hybridisation. S. Piaggi, A. Salvetti, L. Rossi, M. Saviozzi, V. Gremigni, A. Casini, F. Bruschi
- #36            Over expression of Hsp60 in tongue and diaphragm and Hsp70 in thin intestine of rat infected with *Trichinella spiralis*. Ma. G. Basurto Frausto, B. Luna Sánchez, A. Moreno García, O. Y. Barbosa-Cisneros, S. H. Sánchez-Rodríguez

- #39 Effect of extract *Usnea florid* lichen on the implantation and fecundity of adult female Balb/c mouse infected with *Trichinella spiralis*. E. Jiménez-Cardoso, M.L. Caballero-García, R. Mateo-Gonzales, R.M. Chapa-Ruiz, E. Angeles-Angiano

10:15-12:15 am

**Oral Presentations**

- #38 Distribution of *Trichinella britovi* larvae in muscles from experimentally infected foxes (*Vulpes vulpes*). A. Marinculic, R. Beck, D. Miheli, E. Pozio, K. Sever, J.Risti
- #27 Effects of fox, pig, sheep, and poultry bile and non-protein fraction of bile on the *in vitro* survival of domestic and sylvatic species of *Trichinella sp.* G. Theodoropoulos, M. Prokou, V. Georgiadou, M. Petrakos, P. Webster, C.M.O. Kapel
- #37 The virulence of *T. spiralis* is due of its adaptation mechanisms conferred by the heat shock proteins 25 and 90. B. Luna Sánchez, Ma. G. Basurto Frausto, A. Moreno García, O. Y. Barbosa Cisneros, S. H. Sánchez-Rodríguez
- #28 Role of satellite cells in nurse cell formation. Y. Takahashi, T. Boonmars, Z. Wu, I. Nagano
- #33 Fusion and differentiation in mammalian skeletal muscle cells that express *Trichinella spiralis* p43. D. P. Jasmer, X. Cheng, D. Kwak
- #35 Detection of syndecan-1 in muscle cells infected with *Trichinella spiralis*. D.P. Beiting, P.W. Park, J.A. Appleton
- #52 Are bacillary bands responsible for expulsion of *Trichinella spiralis*? W. J. Kozek

12:15-1:30 pm

**Lunch**

**Theme - *Trichinella in the host***

**Session IV – Immunity.** L. Yepez-Mulia and Lj. Sofronic (Chairs)

- 1:30-2:00 pm H.R.P. Miller - “Epithelial and mast cell interactions in the effector response against adult *Trichinella spiralis*”
- 2:00-2:30 pm J. Appleton - “Immunity to the muscle stage”
- 2:30-3:30 pm **Poster Presentations**

- #41 Chemokine changes in mast cells stimulated by TSL-1 antigens. S. Lugo-Hernández, E. García-Zepeda, M. Ramírez, G. Ortega-Pierres, N. Arizmendi-Puga, L. Yèpez-Mulia

- #43 Expression of *Trichinella spiralis* DNA vaccine in mammalian cells. J. Cui, Z.Q.Wang, H.M. Han, R.L.Li
- #44 Expression of *Trichinella spiralis* DNA vaccine in skin and muscle of BALB/c mice. J.Cui, Z.Q.Wang, H.W.Zhang, B.L.Xu
- #45 Vaccination of mice with DNA vaccine induces immune response and protection against *T. spiralis* infection. Z.Q.Wang, J.Cui, H.M. Han, H.Y. Wei, H.W. Zhang, R.L.Li
- #46 Influence of adjuvant formulation on induced host protection in a mouse vaccination model against *Trichinella spiralis*. S. Deville, A. de Pooter, V. Lainé-Prade, M. Cote, S. Ascarateil, J. Aucouturier, P. Boireau, I. Vallée
- #48 A strong antibody response against a 49 kDa antigen of *Trichinella spiralis* newborn larva. M.R. Salinas-Tobón, A. Navarrete-Leon, J. Hernández-Sánchez
- #49 Newborn larva antigen recognition during *Trichinella spiralis* infection. M.R. Salinas-Tobon, M.R. Epitacio, B.E. Mendez-Loredo, D. Esquivel-Aguirre, D.M. Martinez-Abrajan, J. Hernandez-Sanchez
- #55 Analysis of the permanence of antibodies against *Trichinella spiralis* in the offspring of mothers infected with the parasite. C. Maldonado-Tapia, G. Reveles-Hernández, S. Saldívar-Elías, J. Muñoz-Escobedo, A. Moreno-García
- #56 Evaluation of the protection induced by four immunogens against *Trichinella spiralis* infection in experimental trichinellosis. A. Moreno-García, R. Roman-Díaz, E. García-Mayorga, G. Reveles-Hernández, J. Muñoz-Escobedo

3:30-5:30 pm

**Oral Presentations**

- #54 IL-10 prevents liver necrosis during murine infection with *Trichinella spiralis*. S. K. Bliss, A. Alcaraz, J. A. Appleton
- #47 Production of antibodies and expression of cytokines mRNA in pig intestinal mucosa during *Trichinella spiralis* infection. M. Picherot, M. Cote, K. Noeckler, F.J. Serrano, F. Le Guerhier, I. Oswald, P. Boireau, I. Vallée
- #51 Kinetics of antigen recognition by antibodies produced at the intestinal level in mice infected with *Trichinella spiralis*. P.B. Garcia-Reyna, R. Fonseca-Liñan, L. Yepez-Mulia, R. Salinas-Tobón, I. Vallé, P. Boireau, M.G. Ortega Pierres
- #50 *Trichinella spiralis* glycans complexed with monoclonal IgG isotypes interact with mast cell Fc receptors. S. Thrasher, D. Holowka, and J. Appleton

- #40 IgE enhances clearance of *Trichinella spiralis* and regulates mast cell responses in mice. M.F. Gurish, P. Bryce, H. Tao, A.B. Kisselgof, E. Thornton, H.R.P. Miller, K.F. Austen, D. Friend, H. C. Oettgen
- #42 TSL-1 antigens and substance P activate mast cells in a similar manner. N. Arizmendi, J.A. Enciso, G. Ortega Pierres, D. Befus, L.Yépez-Mulia
- #53 The macrophage mannose receptor involvement in the innate immune response to the infection with parasite *Trichinella spiralis*. A. Gruden-Movsesijan, Lj. Sofronic Milosavljevic
- #46 Influence of adjuvant formulation on induced host protection in a mouse vaccination model against *Trichinella spiralis*. S. Deville, A. de Pooter, V. Lainé-Prade, M. Cote, S. Ascarateil, J. Aucouturier, P. Boireau, I. Vallée

### **Wednesday August 11, 2004**

**Session V** - Emergence in human and animal populations. M. van der Giessen and L.-M. Yuan (Chairs)

- 8:30-9:00 am N. Ozeretskovkaya and K.D. Murrell - “New trends in the epidemiology and clinical patterns of human trichinellosis in Russia at the beginning of the XXI century”
- 9:00-9:30 am I. Owen - “*Trichinella papuae* in humans and animals of Papua New Guinea”
- 9:30-10:15 am **Poster Presentations**
- #57 Viability of *Trichinella* larvae outside of the host’s body in different environmental conditions. M.Mahdavi, J.Massoud
- #59 The influence of a high prevalence of sylvatic trichinellosis on the domestic dog population in Finland. L. Oivanen, A. Näreaho, S. Jokela, U. Rikula, R. Gamble, A. Sukura
- #61 Outbreak of trichinellosis associated with consumption of walrus in West Greenland. L.N. Møller, E. Petersen, C.M.O. Kapel, M. Melbye, A. Koch
- #62 Human trichinellosis in Greenland. L.N. Møller, S. Andersen, M. Melbye, E. Petersen, C.M.O. Kapel, P. Laurberg, A. Koch
- #64 *Trichinella nativa* in a black bear from Plymouth, New Hampshire. D.E. Hill, H.R. Gamble, D.S. Zarlenga, C. Coss, J. Finnigan
- #67 Trichinellosis of wild mammals in northwest Ukraine. I. A. Akimov, J.M. Didyk, I. I. Schmalhausen
- #69 Natural and synanthropic *Trichinella* infection in the Central Region of Russia. O.N. Andreyanov, A.S. Bessonov,

- #70 The prevalence of *Trichinella britovi* among different populations of wolves in Croatia. R. Beck, J. Kusak, A. Marinculic, D. Huber, A. Beck, E. Pozio, G. Marucci

10:15-12:00 am

**Oral Presentations**

- #58 Epidemiology of *Trichinella* in wildlife in the Netherlands and the first isolation of *T. pseudospiralis*. J.W.B. van der Giessen, M. Fonville, A. de Vries, I. Briels, M. van Eckerveld, P. Teunis
- #63 Molecular epidemiology of *Trichinella* spp. in three Baltic countries: Lithuania, Latvia and Estonia. A. Malakauskas, V. Paulauskas, P. Keidans, T. Järvis, C. Eddi, C.M.O. Kapel
- #68 Trichinellosis in wild and domestic animals in Poland. W. Cabaj, B. Moskwa, K. Pastusiak, J. Bien, A. Malczewski
- #66 The occurrence and distribution of *Trichinella* spp. in Canadian wildlife. A. Gajadhar, L. Forbes, T. Steeves-Gurnsey
- #60 *Trichinella britovi* in sylvatic carnivores of Guinea Conakry (West Africa). E. Pozio, P. Pagani, G. Marucci, L. Rossi, G. La Rosa
- #65 The evolution of trichinosis in men studied on necroptical data (Summary). D. Cristea, E. Cristea, Gh. Cristea

12:00-1:15 pm

**Lunch**

**Session VI - Human disease and treatment: J. Dupouy-Camet and F. Bruschi (Chairs)**

1:15-3:00 pm

**Oral Presentations**

- #80 An algorithm for diagnosing an acute *Trichinella* infection. J. Dupouy-Camet, F. Bruschi
- #83 Heart specific antigens recognized by trichinellosis patient sera. F. Bongiorno, S. Tommasi, S. Mazzoni, P. Migliorini, F. Bruschi
- #75 Cell-mediated immune response to *Trichinella* in persons with a old history of trichinellosis. M.A. Gomez Morales, A. Ludovisi, E. Pozio
- #71 Anthelmintic activity of a novel 2-(trifluoromethyl) benzimidazole derivative in experimental trichinellosis. J. González, O. Soria, I. V. Cruz, A. Hernández-Campos, F. Hernández-Luis, R. Castillo, L. Yépez-Mulia
- #76 Improvement of the albendazole efficacy against encapsulated larvae of *Trichinella spiralis* in a murine model using a Hydroxypropyl- $\beta$ -Cyclodextrin liquid formulation. M.A. Gomez Morales, A. Casulli, E. Pozio

- #86 Evaluation of three anthelmintics on intestinal and muscular phase infection of *Trichinella spiralis* in the pig model. I. Chavez Ruvalcaba, G. Reveles-Hernández, S. Saldivar- Elias, J. Muñoz- Escobedo, A. Moreno-García
- #81 Persistence of reactivity against the 45 kDa glycoprotein (45 gp) in late trichinellosis patients. F. Bruschi, M.T. Locci, W. Cabaj, B. Moskwa, B. Castagna, W. Kociecka, M. Masetti
- Poster Presentations** (available for viewing from 9:30-10:15)
- #72 Human trichinellosis: presence of specific IgE and IgG<sub>4</sub> in sera from patients undergoing the acute and chronic phases of the infection. M.A. Calcagno, M.A. Forastiero, M.L. Verzoletti, S.N. Costantino, S.M. Venturiello
- #73 Human trichinellosis during pregnancy: a case report. S.M. Venturiello, G.G. Nuñez, M.I. Calvo, S.N. Costantino, M. Derdoy, T. Gentile
- #74 Treatment with albendazole (Eskazole) in trichinellosis. R. Olariu, L. Negrutiu, I. Iacobiciu, G. Darabus, A. Koreck, I. Marincu
- #77 The concept of primary and secondary trichinellosis. G. Enache, D. Panaitescu
- #78 Newborn with trichinellosis. G. Enache, D. Panaitescu
- #79 Trichinellosis and diabetes. G. Enache, D. Panaitescu
- #82 Re-evaluation after 15 years of patients involved in a trichinellosis outbreak caused by *Trichinella britovi*. D. Piergili-Fioretti, B. Castagna, O. Vittori, D. Frondizi, R. F. Frongillo, F. Bruschi
- #84 Rehabilitation of Trichinellae. V. A. Britov, E. A. Nivin, I. N. Lukashkova
- #85 Evaluation of albendazole in intestinal and muscular phase infection by *Trichinella spiralis* in a murine model. A. Moreno-García, G. Reveles-Hernández, I. Chávez- Ruvalcaba, J. Muñoz- Escobedo
- #87 Effect of albendazole in pregnant rats infected with *Trichinella spiralis*. G. Reveles-Hernández, S. Saldivar- Elias, A. Moreno-García, J. Muñoz- Escobedo
- #88 An immuno-polymerase chain reaction assay for circulating antigens in trichinellosis. L.Hui, X.Bianli, Z.Xudong, D.Yan

**3:00 pm**

**ICT Business Meeting**

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**Thursday August 12, 2004**

**Theme - Protecting Public Health**

**Session VII - Food animals: epidemiology and food safety.** L. Oivanen and A. Gajadhar (Chairs)

8:30-9:00 am K. Cuperlovic - "Reemergence of trichinellosis southeast Europe due to political and economic changes"

9:00-9:30 am L. Forbes - "Food safety risks associated with trichinellosis in marine mammals"

9:30-10:15 am **Poster Presentations**

- #89 Detection of anti-*Trichinella* antibodies in chronically infected horses by IFA and Western blot, but not by ELISA. Lj. Sofronic Milosavljevic, N. Ilic, M. Djordjevic, M.Savic, A. Gruden-Movsesijan, K. Cuperlovic, K.D. Murrell
- #90 Trichinellosis in Argentina: an historical review. M. Ribicich, H.R. Gamble, J. Bolpe, A. Rosa, A. Franco
- #91 Epidemiological investigation for the identification of a trichinellosis focus. R. Olariu, L. Negrutiu, G. Darabus, I. Iacobiciu, A. Koreck, I. Marincu
- #94 *Trichinella nativa* in experimentally infected seals. C.M.O. Kapel, L. Measures, L. Moeller, L. Forbes, A. Gajadhar
- #95 Epidemiology: frequency of *T. spiralis* in horses from two slaughter house (Municipal and rural) in the State of Mexico. E. Jimenez-Cardoso, M.L. Caballero García, E. Trejo-Hernández, G. Uribe-Gutiérrez, F.R. Gay-Jiménez
- #96 The epidemiology of trichinosis in the Jiu Valley in the 1987-2003 period (Summary). D. Cristea, E. Cristea
- #97 The epizootology of trichinosis in the Jiu Valley in the 1988-2003 period (Summary). Gh. Cristea, E. Cristea, D. Cristea
- #100 Effects of social-economic factors on epidemic process at *Trichinella spiralis* infection in Russia. A.S. Bessonov
- #101 Survey on porcine trichinellosis in Ecuador. M.A. Chávez-Larrea, P. Dorny, L. Moeller, W. Benítez-Ortiz, M. Barrionuevo-Samaniego, R. Rodríguez-Hidalgo, J. Ron-Román, F. Proaño-Pérez, B. Victor, J. Brandt, C. Kapel, J. de Borchgrave
- #102 Trichinosis in Armenia. A. Asatryan, A. Zanginyan, M. Harutunyan, L. Ghazaryan, A. Nerkararyan

- #104 Checking the accuracy of trichinelloscopy in naturally infected pigs with low muscle larvae burden. R. Beck, Ž. Mihaljević, A. Marinculic
- #105 Detection of *Trichinella spiralis* in Zacatecas, Mexico. J. Muñoz- Escobedo, G. Reveles-Hernández, S. Saldivar- Elias, A. Moreno-García
- #123 The status of trichinellosis in Uzbekistan. M. Aminjonov

10:15-12:00 am

**Oral Presentations**

- #93 Epidemiology of equine trichinellosis: the risk from animal protein feeding practices. K.D.Murrell, M. Djordjevic, K. Cuperlovic, Lj. Sofronic, M. Savic, S. Damjanovic
- #103 Risk for *Trichinella* infection in Romanian horses. C-M Cretu, I. Dida, K. Nockler, E. Pozio, C. Kapel and P. Boireau, C. Davila
- #92 Experimental studies in SPF pigs on *Trichinella* detection in different diagnostic matrices. K. Noeckler, F. J. Serrano Aguilera
- #98 *Trichinella pseudospiralis* from a wild pig in Texas, USA. H. R. Gamble, E. Pozio, J.R. Lichtenfels, D. S. Zarlenga
- #99 Epidemiological investigation of *Trichinella* spp in wild boars in Croatia. S. Bosnić, A. Marinculic, M. BeniĆ, R. Beck
- #106 Evaluation of ELISA for detection of *Trichinella* antibodies in muscle juice samples of naturally infected pigs. R. Beck, A. Gašpar, Ž. Mihaljević, A. Marinculic, D. Stojčević, M. Brstilo
- #107 Meat juice of infected pigs as a source for specific *T. spiralis* antibody detection. Lj. Sofronic Milosavljevic, M. Petrovic, M. Djordjevic, M. Savic, K. Cuperlovic, I.V. Patrascu

12:00-1:15 pm

**Lunch**

**Session VIII** - Surveillance, control and legislation. R. Gamble and K. Noeckler (Chairs)

- 1:15-1:45 pm C. Kapel - “Changes in EU legislation on inspection and surveillance”
- 1:45-2:15 pm D. Pyburn - “Trichinae certification in the United States Pork Industry”

2:15-3:00 pm

**Poster Presentations**

- #108 Study concerning pathomorphological aspects in larvae and cysts of *Trichinella spiralis* in swine meat. I. Cironeanu



- #111 Detection of *Trichinella* infection in field pigs in Argentina using the ELISA test. M. Ribicich, H. R. Gamble, A. Rosa, I. Sommerfelt, J. Bolpe, H. Torno, M. Verdier, A. Franco
- #112 On fundamental problems of trichinellosis in man and animals in Romania. Gh. Olteanu, I. Cironeanu
- #114 Intensity of *Trichinella* sp. infection in the pig. Gh. Cristea, D. Cristea, E. Cristea,
- #115 The French National Reference Center on *Trichinella*. J. Dupouy-Camet, T. Ancelle
- #116 Necessity for the application of quality assurance (QAS) and proficiency samples programs in meat inspection for trichinellosis. M. Djordjevic, K. Cuperlovic, M. Savic, S. Pavlovic
- #118 Comparison of two antigens for demonstration of *Trichinella* spp. antibodies in blood and muscle fluid of foxes, pigs and wild boars. L.N. Møller, E. Petersen, H.R. Gamble, C.M.O. Kapel
- #121 Common antigens among *T. spiralis*, *P. westermani* and *C. sinensis*. Z.Q. Wang, J. Cui, D. Zhang

3:00-4:30 pm

**Oral Presentations**

- #109 An accreditation program for reliable *Trichinella* testing of pork and horsemeat by private industry in Canada. W. B. Scandrett, L. B. Forbes, A. A. Gajadhar
- #110 A control program to reduce the risk of infection with *Trichinella spiralis* in New Zealand pigs. E.K.B. Richardson, D.E. Lawton, M.A. Potter
- #117 Successful eradication of swine trichinellosis in highly endemic village in Croatia. A. Marinculic, R. Beck
- #119 Comparison of two iELISA procedures for early detection of specific *Trichinella* antibodies. W. Cabaj, B. Moskwa, J. Bien, K. Pastusiak, J. Pourquier, K. Nöeckler, F. J. Serrano, E. Pozio
- #120 Specific diagnostic antigens in ES products from *T. spiralis* muscle larvae. Z.Q.Wang, J.Cui, D.Zhang, H.Y. Wei, B.L.Xu

4:30 pm

**Closing Ceremony**

# Social Events Schedule

## Sunday, August 8, 2004

6:00 PM	Opening Ceremony	Manchester Conference Center, USD
7:00 PM	Reception	Manchester Conference Center, USD
9:00 PM	Accompanying Persons Meeting	Manchester Conference Center, USD

## Monday, August 9, 2004

7:00 PM	Lecture on Old Town San Diego	Casa Guadalajara, Old Town
8:00 PM	Fiesta Buffet	Casa Guadalajara, Old Town

buses depart Manchester Conference Center at 6:15 and 6:30 PM  
buses return to Manchester Conference Center at 10:45 and 11:00 PM

## Tuesday, August 10, 2004

6:30 PM	Reception	Birch Aquarium, La Jolla
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buses depart Manchester Conference Center at 6:00 PM  
buses return to Manchester Conference Center at 10:00 PM

## Wednesday, August 11, 2004

6:30 PM	Dinner cruise	San Diego Harbor
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buses depart Manchester Conference Center at 6:00 PM  
buses return to Manchester Conference Center at 10:00 PM

## Thursday, August 12, 2004

6:30 PM	Beach party	Vacation Island, Mission Bay
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buses depart Manchester Conference Center at 5:45 PM  
buses return to Manchester Conference Center at 10:30 PM

## Invited Speakers:

### Keynote

**Maintaining public confidence in the face of global threats to animal health, public health and food security.** Brian Evans, Chief Veterinary Officer for Canada, Canadian Food Inspection Agency

The current threat environment to public and animal health and food safety arising from globalization, human population demographics, pathogen adaptation, agro-terrorism and other factors demands that approaches to prevention, preparedness, response and recovery extend seamlessly across the animal health and public health community and beyond national borders. Fortunately, the potential for, and consequences of, accidental, incidental or deliberate introduction of animal, zoonotic or food safety pathogens risks in North America have been recognized for a number of years by regulatory officials. As a consequence, investments have been made over the past number of decades that provide a foundation for an integrated and expanded capacity. Regrettably not all sectors or levels of the animal and public health community are yet engaged. It is also recognized that the investments made to date have been modest in comparison to the magnitude of the challenge. Of paramount importance to ensuring that the impacts of a significant food safety, public or animal health event can be managed in a responsive and responsible manner is the required additional investments that must be made in three key areas: effective risk communication to maintain public trust and consumer confidence; creating the environment that will minimize the social and economic consequences of a finding; and establishing a seamless public and animal health community.

### Presidential Address

**The broad spectrum of *Trichinella* hosts: from cold- to warm-blooded animals.** E. Pozio, Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Rome, Italy

In recent years, studies have shown that the host range is wider than previously believed, and new *Trichinella* species and genotypes have been described. Three classes of vertebrates are known to act as hosts (i.e., mammals, birds, and reptiles), and infected vertebrates have been detected in all continents but Antarctica. Mammals represent the most important hosts, and all *Trichinella* species are able to develop in this vertebrate class. Natural infections with *Trichinella* have been described in more than 150 mammalian species belonging to 10 orders (i.e., Marsupialia, Insectivora, Edentata, Lagomorpha, Rodentia, Cetacea, Carnivora, Perissodactyla, Artiodactyla, and Primates). The epidemiology of the infection greatly varies by species, in relation to characteristics such as diet, life span, distribution, behavior, and relationships with humans. The non-encapsulated species *T. pseudospiralis*, detected in both mammals (14 species) and birds (13 species), shows a cosmopolitan distribution with three distinguishable populations in the Palearctic, Nearctic and Australian region. Two additional non-encapsulated species, *T. papuae*, detected in wild pigs and saltwater crocodiles of Papua New Guinea, and *T. zimbabwensis*, detected in farmed Nile crocodiles and in sylvatic monitor lizards of Zimbabwe, can complete their life cycle in both mammals and reptiles. To the best of our knowledge, *T. papuae* and *T. zimbabwensis* are the only two parasites known to complete their entire life cycle independently of whether the host is warm-blooded or cold-blooded. This indicates that these two *Trichinella* species are capable of activating different physiological mechanisms, according to the specific vertebrate class hosting them. Work funded, by the EU project "TRICHIPORSE" (contract QLK1-CT-2001-01156).

**Discovery of candidate developmentally expressed genes in *Trichinella spiralis*.** M. Mitreva<sup>1</sup>, D.P. Jasmer<sup>2</sup>, J. Appleton<sup>3</sup>, J. Martin<sup>1</sup>, M. Dante<sup>1</sup>, T. Wylie<sup>1</sup>, S. W. Clifton<sup>1</sup>, R. H. Waterston<sup>1,4</sup>, and J. P. McCarter<sup>1</sup>, Genome Sequencing Center, Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63108<sup>1</sup>, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040<sup>1</sup>, James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853<sup>3</sup>, Department of Genome Sciences, University of Washington, Seattle, Washington 98195<sup>4</sup>

To identify genes expressed by parasites during a variety of life-cycle stages and at different steps during host-parasite interaction we have constructed stage-specific libraries and generated expressed sequence tags (ESTs) from 28 parasitic nematode species. 10,130 ESTs originate from the adenophorean nematode *Trichinella spiralis*. Representation of multiple life-stages was achieved by sequencing cDNA libraries constructed from immature L1, muscle larvae and adult. We will report on the progress of the *Trichinella spiralis* EST analysis including: creation of NemaGene clusters to reduce sequence redundancy, identification of common and rare represented genes, identification of stage-biased expression, functional classification based on Gene Ontology and KEGG assignments and signal peptide for secretion, and identification of genes orthologues in *C. elegans* and other nematodes. All sequences are publicly available at [www.ncbi.nlm.nih.gov/dbEST](http://www.ncbi.nlm.nih.gov/dbEST). The project is funded by NIH-NIAID Research Grant AI 46593.

**The biology of *Trichinella*: what we still need to know.** Despommier, D.D., Department of Environmental Health Sciences, Columbia University, New York City, New York 10032

The biology of *Trichinella spiralis* was comprehensively reviewed last in 1983. While much progress toward understanding the mechanisms employed by this worm to carry out its life in its mammalian host has been made since then, nonetheless, the life of *Caenorhabditis elegans* remains the best-studied example of a nematode. This is unfortunate, since the latter offers little in the way of insight as to the mechanisms used by its parasitic relatives to withstand the onslaught of host immune responses, and, at the same time, grow to adulthood and reproduce. The adult and infective muscle larva of *T. spiralis* possess a stichosome composed of a row of specialized cells that synthesize and then selectively secrete hundreds of novel proteins specific to each stage, the function of which remain largely undefined. Unraveling the role(s) they play in aiding the parasite in its quest to maintain itself in niches within columnar cells of the small intestine and a portion of striated skeletal muscle cell is a challenge rich with the promise of discovering new classes of therapeutic agents that include: immune inhibitors, inducers of angiogenesis, inducers of collagen synthesis, and regulators of host-specific genes, for example.

**A potential role for *Trichinella spiralis* secreted proteins in modulation of host purinergic signalling.** K. Gounaris, Department of Biological Sciences, Biochemistry Building, Imperial College London, London SW7 2AZ, UK

Extracellular nucleotides are signalling molecules which modulate a wide variety of physiological responses in mammalian tissues, and are activators of the innate immune system. The process by which nucleotides exert their effect is termed purinergic signalling and it relies on stimulation of nucleotide release, their metabolism by enzymes acting in an extracellular manner, and the presence of receptors which selectively bind the resulting products and mediate signal transduction. *Trichinella spiralis* secretes a variety of enzymes which utilise and/or metabolise extracellular nucleotides. We have identified protein kinase(s), a nucleotide diphosphate kinase, a 5'-nucleotidase and an adenosine deaminase among the secreted proteins of this parasite. Our data indicate that these enzymes modulate the type and concentration of extracellular nucleotides. They thus have the potential to regulate purine/pyrimidine receptor activation and interfere with host purinergic signalling and resultant inflammatory responses.

**Epithelial and mast cell interactions in the effector response against adult *Trichinella spiralis*.**

HRP Miller, PA Knight, AD Pemberton, J Brown, SH Wright and EM Thornton, University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, EH 25 9RG, UK

After establishing in the gut, *T spiralis* provokes a potent protective immune response, which, in certain strains of rodents, eliminates adult worms within 10-14 days. The protective mechanisms are complex, involving T and B cells with TH2 mediated recruitment of bone marrow-derived effector cells, including mast cells, basophils and eosinophils. The adult worms establish within their intraepithelial niche and there are associated changes in enterocyte kinetics and differentiation. Some of these changes are, similarly, T cell-regulated. We have tested the hypothesis that intestinal mucosal mast cells (MMC) and enterocytes are mutually interactive and that they function in concert in the elimination of the worms. A targeted disruption of the gene encoding the major MMC granule  $\beta$ -chymase, mouse mast cell protease-1 (mMCP-1), results in enhanced and, as in wild type controls, >80% intraepithelial, MMC recruitment. But there is reduced ability to expel worms, pointing to the importance of this chymase in the effector response. The hypothesis was further explored in the knowledge that mMCP-1 expression *in vitro* is highly TGF- $\beta_1$ -dependant and that the enterocyte-expressed integrin,  $\alpha V\beta_6$ , activates latent TGF- $\beta_1$  on the cell surface. In mice lacking  $\alpha V\beta_6$  integrin, MMC recruitment is enhanced but with less than 10% intraepithelial MMC. As predicted, in the absence of activated TGF- $\beta_1$ , there is little or no expression of mMCP-1. Preliminary analysis shows that worm expulsion is also compromised. These data support the hypothesis that there is MMC/enterocyte interaction in the elimination of infection. The mechanisms involved will be discussed. *Supported by the Wellcome Trust (grant #060312).*

**Immunity to the muscle stage.** D.P. Beiting and J.A. Appleton, James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

The life cycle of *Trichinella* is completed when first-stage larvae mature in striated muscle. Larvae resident in muscle are immunogenic, as evidenced by sustained antibody production against larval glycans. In contrast to the systemic immune response to muscle infection, local inflammation is limited. Control of local inflammation promotes the survival of the parasite while limiting disease in the host. The nature of this response is shared across host species and among related species of the parasite. Non-encapsulated species of *Trichinella* induce little to no local inflammatory response in reptilian and mammalian hosts. Encapsulated species of *Trichinella* induce a limited local inflammation in a variety of mammalian hosts. We have investigated the mechanisms that control local inflammation in mice infected with *T. spiralis*. Synchronous muscle infections were established by injecting newborn larvae intravenously into C57BL/6 mice that were deficient in IL-10. We found that IL-10 limited local inflammation during the acute stage of muscle infection with *T. spiralis*, while the control of inflammation during chronic infection was independent of IL-10. Larvae developed and survived in the presence of the dysregulated inflammatory response, demonstrating that *T. spiralis* is well adapted to its host.

**New trends in the epidemiology and clinical patterns of human trichinellosis in Russia at the beginning of the XXI century.** N.N.Ozeretskovskaya<sup>1\*</sup>, L.G.Mikhailova<sup>2</sup>, T.P.Sabgaida<sup>1</sup>, A.S.Dovgalev<sup>3</sup>. <sup>1</sup>E.I.Martsinovsky Institute of Medical Parasitology and Tropical Medicine, Moscow, Russia, <sup>2</sup>Department of the State Epidemic Surveillance, Ministry of Health of the Russian Federation, <sup>3</sup>Moscow regional Centre of Epidemic Surveillance, Moscow, Russia

The official statistics reflected the gradual decline of the incidence of trichinellosis in Russia from 971 cases in 1996 to 527 cases in 2003. Among the total 864 cases in 47 trichinellosis outbreaks in 1998-2002 35.8% were due to infected pork (80% in 1995-1996), 39.5% to infected bear meat, 12.8% to other wild animals meat (e.g. 10.6% badger meat), 11.9% to the game and stray dogs meat. Children composed 18.4% of infected from pork, 14.7% from bear-, 31.5% from badger-, 14.6% from dog-meat. In total, 81.0% of pork-cases belonged to the European part of the country, 89.4% of bear-cases to the Asian part where 71.7% of badger- and 90.3% of dog-cases were originated from. The percent of severe cases of disease from pork and bear meat was 7.7 and 7.9, respectively. Regardless of the geographic region incubation period in bear-meat outbreaks was about 30 days that permitted a partial preventive mebendazole therapy. Four single lethal cases from the bear meat occurred in the aboriginal of Siberia and one case from pork in the European part. Severe cases from badger and dog meat was revealed in 1.1 and 1.9%, respectively. Badger infection in Siberia was less serious than in the European part. The contributing factors of the slow decline of the incidence of trichinellosis in Russia and the rise of zoonotic infection are the distribution and consumption of veterinary uncontrolled pork, poaching and distribution of wild animals meat. The demographic changes (i.e. migration) lead to the loss of basic folk food habits, the neglect of medical and civil regulations. These trends should be seriously evaluated by the Departments of Health, Education, Culture, and by the Veterinary service.

***Trichinella papuae* in humans and animals of Papua New Guinea.** I.L. Owen<sup>1</sup>, M.A. Gomez Morales<sup>2</sup>, G. La Rosa<sup>2</sup>, G. Marucci<sup>2</sup>, E. Pozio<sup>2</sup>, <sup>1</sup>c/o National Veterinary Laboratory, National Agriculture Quarantine & Inspection Authority, PO Box 741, Port Moresby, Papua New Guinea; <sup>2</sup>Department MIPI, Istituto Superiore di Sanità, Rome, Italy

*Trichinella papuae*, first described in 1999, was recovered from wild and domestic (village) pigs in one remote locality (Bensbach) of SW region of Papua New Guinea (PNG). The aim of the present work was to investigate the epidemiology of *T. papuae* in animals and humans in PNG. A sero-epidemiological survey carried out on 1,536 persons from 51 villages in Morehead District, showed a prevalence for *Trichinella* antibodies ranging from 0 up to 40%. In the Bensbach region, the higher the prevalence, the shorter the distance of the villages from the hunting area (Bula Plain), where 11.5% (9/78) of wild pigs were positive for *Trichinella*. However, no person with a positive serology showed clinical signs pathognomonic for trichinellosis. Due to the infectivity of *T. papuae* for both mammals and experimentally infected reptiles, the presence of this parasite in saltwater crocodiles (*Crocodylus porosus*) in PNG was investigated. Of 150 crocodiles examined, 24 animals (16.1%), all from Kikori, Gulf Province, were positive for the non-encapsulated larvae of *T. papuae*. The sequence analysis of the region within the large subunit mitochondrial DNA, known as the expansion segment V, revealed the presence of a genetic polymorphism between *T. papuae* isolates from 2 different provinces of PNG, which will be useful to trace back the geographical origin of an infected animal. This is the second report of a natural infection of reptiles with *Trichinella*. The epidemiology of the infection in crocodiles could be related to the practice of local people to feed young crocodiles, caught in nature, with wild pig meat before they are sold to a crocodile farm.

**Reemergence of trichinellosis in southeast Europe due to political and economic changes.** K. Cuperlovic, M. Djordjevic, S. Pavlovic, Institute for Meat Technology and Hygiene, Serbia and Montenegro, 11000 Beograd. Kacanskog 13

Social, economic and political factors responsible for reemergence of trichinellosis in southeast Europe countries are discussed in the communication. Southeast European countries from geographical point of view comprise Balkan region and bordering countries, including Albania, Bulgaria, Bosnia and Herzegovina, Croatia, Greece, Hungary, Macedonia, Romania, Serbia and Montenegro, Slovenia, European part of Turkey (5% of the total country). Countries of southeastern Europe occupy very important strategic position and represent a bridge between Europe and Asia. Differences in ethnicity and religion, influences and interests from abroad, different cultural and economic development and many others differences resulted in antagonisms and even wars between these countries. In majority of the southeast European countries cases of trichinellosis among human population and animals were described at late 19<sup>th</sup> or early 20<sup>th</sup> centuries. *Trichinella* infections among wild life were also described in all mentioned countries. Today, the prevalence of trichinellosis is different among Balkan and bordering countries. High prevalence of trichinellosis in domestic animals and human are reported in Bulgaria, Serbia and Montenegro, Romania and Croatia. Medium prevalence was found in Bosnia and Herzegovina. In Hungary, Greece, Slovenia, Macedonia and Turkey trichinellosis is sporadic. Reemergence of trichinellosis is connected with the changes of social and political system in Bulgaria and Romania. In Serbia and Montenegro as well in Croatia, however, reemergence of trichinellosis was not only due to political and social changes but also due to wars that took places in this countries during the last years of previous century.

**Food safety risks associated with trichinellosis in marine mammals.** L.B. Forbes, Centre for Animal Parasitology, Canadian Food Inspection Agency, Saskatoon, SK, Canada S7N 2R3

Trichinellosis in arctic wildlife was first confirmed in 1934 in foxes and polar bears, and human outbreaks were generally attributed to the consumption of meat from these species. In 1947, an outbreak of human trichinellosis in Greenland was linked to the consumption of walrus meat, and extensive follow-up surveys identified trichinellosis in walruses and seals. Trichinellosis has since been confirmed in polar bears, walruses, bearded seals, ring seals and a single Beluga whale. Isolates from marine mammals have been characterized as *Trichinella nativa* (T2), and appear identical to T2 found in terrestrial mammals. Animal behavior and disease surveillance data have led to a better understanding of the epidemiology of trichinellosis in marine mammals. Cannibalism is believed to be the primary mechanism for maintaining trichinellosis in polar bears and there is mounting evidence to suggest that an independent *Trichinella* cycle may occur in walruses. Seals and whales appear to be incidental hosts. The consumption of infected walrus meat is the most frequent cause of human trichinellosis in the arctic, and at least two regional public health agencies in Canada have developed systems to obtain post-slaughter samples of walrus meat for testing prior to consumption. The CFIA's Centre for Animal Parasitology has worked closely with these groups to develop an appropriate digestion assay for walrus meat and a training program for local laboratory staff conducting the test. Studies using *Trichinella* infected seal meat to produce a variety of country foods have demonstrated that infective larvae can survive for up to 5 months in some preparations. Although recent work supports much of the anecdotal and circumstantial evidence regarding trichinellosis in marine mammals, additional work on disease prevalence and larval survival in traditionally prepared foods is required to further focus and improve public health initiatives in this area.

**Changes in EU legislation on inspection and surveillance.** C.M.O. Kapel, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark



**Trichinae Certification in the United States Pork Industry.** D.G. Pyburn<sup>1</sup>, H.R. Gamble<sup>2</sup>, E.A. Wagstrom<sup>3</sup>, L.A. Anderson<sup>1</sup>, and L.E. Miller<sup>1</sup>, <sup>1</sup>USDA APHIS, Des Moines, IA, USA, <sup>2</sup>National Academy of Sciences, Washington, DC, USA, <sup>3</sup>National Pork Board, Des Moines, IA, USA

Control of *Trichinella* infection in U.S. pork has traditionally been accomplished by inspection of individual carcasses at slaughter or by post-slaughter processing to inactivate parasites. Declines in prevalence of this parasite in domestic swine during the last thirty years, coupled with improvements in pork production systems, allows pork safety to be documented at the farm level. We report here on a pilot study using on-farm auditing to document good production practices for swine. Knowledge of risk factors for exposure of swine to *Trichinella spiralis* were used to develop an objective audit that could be applied to pork production sites. In a pilot study, 461 production site audits were performed by trained veterinary practitioners. The on-farm audit includes aspects of farm management, bio-security, feed and feed storage, rodent control programs, and general hygiene. In the pilot study, objective measures of these good management practices were obtained through review of production records and a site inspection. Of the 461 production site audits, 450 audits (97.6%) indicated adherence to good management practices and these sites were granted either entry into the program or certification. These sites will be audited regularly on a schedule established by the Trichinae Certification Program Standards. The described trichinae certification mechanism will establish a process for ensuring the quality and safety of animal-derived food products from the farm through slaughter. Uniform standards stating the requirements of this program have been developed. Federal regulations in support of the program are currently being developed.

## Contributed Papers:

1. **A new phylogenetic hypothesis for the genus *Trichinella*.** D. S. Zarlenga<sup>1</sup>, B. Rosenthal<sup>1</sup>, G. La Rosa<sup>2</sup>, E. Hoberg<sup>1</sup>, and E. Pozio<sup>2</sup>, US Department of Agriculture, ARS, ANRI, Beltsville, MD 20705<sup>1</sup>, USA; Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy<sup>2</sup>

From its first description in 1835 through much of the following century, the genus *Trichinella* was considered monospecific; however, recent biochemical, molecular, phenotypic, and crossbreeding experiments are consistent with partitioning the genus into multiple species. Herein, we present the first robust phylogenetic hypothesis for the genus *Trichinella* based on variation in several genetic loci using a comprehensive sample of all ecologically and genetically recognized genotypes. An analysis of variation in nuclear and mitochondrial ribosomal DNA, and mitochondrial COX I DNA indicates strong support for an especially close relationship between the non-encapsulated species, *T. papuae* and *T. zimbabwensis*, and between the freeze-tolerant genotypes, *T. nativa* and *Trichinella* T6, where *T. spiralis* is basal to all encapsulated species. Our hypothesis provides a unique historical perspective on the biogeography and epizootiology of the group, indicating: 1) all members of the genus share a close evolutionary relationship to the exclusion of other known nematodes; 2) capsules evolved once in the history of the genus, and; 3) both the encapsulated and non-encapsulated clades contain species that are now cosmopolitan, as well as species inhabiting more geographically and ecologically restricted ranges. Because, the non-encapsulated and encapsulated species have been uniquely recognized on the basis of genotypic and phenotypic variations, this raises the question of whether they may represent evolutionarily distinct genera. Data presented here are consistent with basal partitioning between the encapsulated and non-encapsulated clades. A history of geographic dissemination and host adaptation will be proposed and implications for taxonomic nomenclature discussed.

2. **Phylogenetic analysis of encapsulated and non-encapsulated *Trichinella* genotypes by studying the 5S rDNA tandemly repeated intergenic region.** J.W.B. van der Giessen<sup>1</sup>, M. Fonville<sup>1</sup>, I. Briels<sup>1</sup>, E. Pozio<sup>2</sup>, National Institute of Public Health and the Environment<sup>1</sup>, PB 1, 3720 BA Bilthoven, Netherlands, Istituto Superiore di Sanità<sup>2</sup>, viale Regina Elena 299, 00161 Rome, Italy

The 5S rDNA intergenic region has been detected as a good target to distinguish 8 *Trichinella* species and genotypes among them. In particular, the 5S rDNA region shows a great difference between the encapsulated species and the non-encapsulated species *T. pseudospiralis*. In the present work, we analyzed the 5S rDNA of two additional non-encapsulated species, named *T. zimbabwensis* and *T. papuae*. The amplification of the tandem repeated of the 5S rDNA intergenic region of the encapsulated species of *Trichinella* resulted in a 751 bp fragment, whereas the 3 non-encapsulated species show a fragment of 800 bp. *T. pseudospiralis* shows an additional fragment of 522 bp, which was previously described. Although the size of the 800 bp PCR fragments of *T. zimbabwensis* and *T. papuae* is similar to that of *T. pseudospiralis*, there are differences in the 5S rDNA intergenic regions among the 3 non-encapsulated species. The phylogenetic analysis of the 5S rDNA intergenic region shows the three non-encapsulated *Trichinella* species clustering together and well separated from the encapsulated species. In addition, a single PCR based method allows to distinguish non-encapsulated and encapsulated species between them.

**3. Amplified Fragment Length Polymorphism (AFLP) shows a high resolution power in detecting genetic variation in *Trichinella nativa*.** T. Mikkonen<sup>1</sup>, J. Koort<sup>2</sup>, J. Björkroth<sup>2</sup> and A. Sukura<sup>1</sup>, Department of Basic Veterinary Sciences<sup>1</sup>, Department of Food and Environmental Hygiene<sup>2</sup>, Faculty of Veterinary Medicine, University of Helsinki, Finland

Due to the difficulties to analyze single larva of the parasite and because of relatively small amount of polymorphic genetic markers in *Trichinella* spp. there is need to find methods for studying population genetics within the genus. We tested AFLP (Amplified Fragment Length Polymorphism) in order to find a way for analysing intraspecific diversity of *Trichinella nativa*. Advantages of AFLP are: 1) the small amount of template DNA needed, 2) no prior knowledge of the nucleotide sequence is required. *Trichinella* spp. from foxes and raccoon dogs from eight distinct areas in Finland were determined by multiplex-PCR as *T. nativa* and compared by AFLP. Genomic DNA (400 ng) from a pool of 10-20 larvae were digested using *Hind*III and *Mse*I restriction endonucleases and ligated with adapter. The combination of *Hind*0 and *Mse*0 primers in pre-amplification and further with, *Hind*-C (IRD800 labelled) and *Mse*-C primers in selective amplification, yielded the best results. PCR products were electrophoresed on a LiCor DNA sequencer in a 7% denaturing acryl amide gel. Rich polymorphism with approximately 40-90 bands in the range of 30-240 bp was found. In the analysis of AFLP profiles, distinct clusters were found but the variability between individual hosts was considerable even between the hosts originating from same area. AFLP seems to be extremely discriminatory and therefore it may be a method detecting variation in *Trichinella nativa* populations. However, the heterogeneous patterns obtained from pooled samples emphasizes the need for developing suitable numerical analysis for epidemiological interpretation.

**4. Relationships between *Trichinella* and host species in Europe.** E. Pozio<sup>1</sup>, F. Serrano<sup>2</sup>, P. Dubinsky<sup>3</sup>, W. Cabaj<sup>4</sup>, R. Blaga<sup>5</sup>, I. Dida<sup>6</sup>, D. Christensson<sup>7</sup>, K. Noekler<sup>8</sup>, G. Marucci<sup>1</sup> and G. La Rosa<sup>1</sup>, Istituto Superiore di Sanità, Italy,<sup>1</sup> University of Extremadura, Spain,<sup>2</sup> Slovak Academy of Sciences, Slovakia,<sup>3</sup> Polish Academy of Sciences, Poland,<sup>4</sup> University of Cluj-Napoca,<sup>5</sup> and University of Bucharest,<sup>6</sup> Romania, National Veterinary Institute, Sweden,<sup>7</sup> Federal Institute for Risk Assessment, Germany<sup>8</sup>

The knowledge of the relationship between *Trichinella* and host species is of great importance for the development of prevention and control programs. With this aim, we collected *Trichinella*-positive samples from domestic pigs and wildlife of 13 European countries. Larvae were identified at the species level by a multiplex-PCR analysis. Of 453 isolates examined, 41 originated from domestic pigs infected with *T. spiralis* (79%), *T. britovi* (19%) and *T. pseudospiralis* (2%); 133 from wild boars with *T. spiralis* (58%), *T. britovi* (39%) and *T. pseudospiralis* (3%); 225 from red foxes with *T. spiralis* (4%), *T. britovi* (95.5%) and *T. spiralis*-*T. britovi* mixed infections (0.5%); and 36 from other sylvatic carnivores (18 wolves; 6 brown bears; 5 lynxes; 4 wild cats; and 3 beech martens) infected with *T. spiralis* (8%), *T. britovi* (89%) and *T. pseudospiralis* (3%). *T. spiralis* is the prevalent species in domestic and sylvatic swine, although in Bulgaria, Slovakia and Romania the prevalence of *T. britovi* in wild boars is higher than that of *T. spiralis*. *T. britovi* is the prevalent species in carnivores (94.6%), followed by *T. spiralis* (5%) and *T. pseudospiralis* (0.4%). *T. nativa* has not been detected in the 13 investigated countries, because it is present at higher latitudes. We are grateful to P. Boireau, C. Cretu, J. Dupouy-Camet, S. Komandarev, B. Koudela, L.M. Madeira de Carvalho, P. Rafter and T. Streter, for their support in the present work. Work funded by the EU project "TRICHIPORSE", contract QLK1-CT-2001-01156.

**5. Freeze tolerance of nine *Trichinella* genotypes in muscle tissue of experimentally infected pigs, horses, wild boars, mice, cats, and foxes.** C.M.O. Kapel<sup>1</sup>, P. Webster<sup>1</sup>, A. Malakauskas<sup>2</sup>, Z. Hurnikova<sup>3</sup> and H.R. Gamble<sup>4</sup>, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark<sup>1</sup>, Lithuanian Veterinary Academy, Kaunas, Lithuania<sup>2</sup>, Parasitol. Inst., Slovak Academy of Science, Kosice, Slovak Republic<sup>3</sup>, US Dept of Agriculture, Beltsville, MD, USA<sup>4</sup>

Infected muscle tissue was obtained from a series of experimental infections in pigs, wild boars, horses, mice, cats and foxes, with the aim to evaluate the cold tolerance of nine well-defined genotypes of *Trichinella*. The samples originated from necropsies performed 5, 10, 20 and 40 weeks post inoculation (p.i.) of the respective host species. Sub-samples of 100g were stored at 5, -5 and -18 °C. After 1, 4 and 8 weeks storage, one sub-sample was taken from each temperature treatment and digested; recovered larvae were inoculated into 5 mice per sample (up to 500 larvae per mouse). Five weeks p.i. mice were killed and digested for recovery of muscle larvae. In meat of pigs and wild boars, no *Trichinella* was able to survive at -18°C for 1 wk; most species showed some survival at -5°C, and all survived at +5°C for 4 wks. In mice, only *T. nativa* and *Trichinella* T6 survived at -18°C for 1 wk, but *T. murrelli* and *T. britovi* showed good tolerance at -5°C. In the carnivores, *T. nativa* and *Trichinella* T6 survived at -18°C for 4 wks; *T. murrelli* and *T. britovi* survived 1 wk at this temperature. In horses, which were only inoculated with *T. spiralis*, *T. britovi* and *T. pseudospiralis* only, all three species survived at both -5°C and -18°C for 1, 4 and 8 wks. The results clearly show that freezing at -18°C is an effective way to inactivate *Trichinella* in pork and wild boar meat, but that some *Trichinella* genotypes survive freezing in meat of carnivores. Further, it appears that horse meat most likely contains substances that effectively prevent freezing of *Trichinella* muscle larvae.

**6. Muscle distribution of sylvatic and domestic *Trichinella* larvae in production animals and wildlife.** C.M.O. Kapel<sup>1</sup>, P. Webster<sup>1</sup> and H.R. Gamble<sup>2</sup>, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark<sup>1</sup>, US Department of Agriculture, Beltsville, Maryland, USA<sup>2</sup>

Only a few studies have compared the muscle distribution of the different *Trichinella* genotypes. In this study, data was obtained from a series of experimental infections in pigs, wild boars, foxes and horses, with the aim to evaluate the predilection sites of nine well-defined genotypes of *Trichinella*. Necropsy was performed at 5, 10, 20 and 40 weeks post inoculation. From all host species, identical muscles/muscle groups were examined by artificial digestion. In the foxes, where all *Trichinella* species established in high numbers, the encapsulating species were found primarily found in the tongue, extremities and diaphragm, whereas the non-encapsulating species had predilection site in the diaphragm. In pigs and wild boars, only *T. spiralis*, *T. pseudospiralis* and *T. nelsoni* showed persistency of muscle larvae (ML), but for all genotypes the tongue and the diaphragm was found to be predilection sites. This tendency was most obvious in light infections. In the horses, *T. spiralis*, *T. britovi*, and *T. pseudospiralis* all established at high levels, with predilection sites in the tongue, the masseters and the diaphragm. For all host species, high ML burdens appeared to be more evenly distributed with less obvious predilection than in light infections: predilection site muscles harboured a relatively higher percent of the larval burden in light infections than in heavy infections. This probably reflects increasing occupation of available muscle fibres. Predilection sites appear primarily to be influenced by host species and secondly by the age and level of infection.

**7. Comparative infectivity of three *Trichinella* species in ponies.** C.M.O. Kapel<sup>1</sup>, Z. Hurnikova<sup>2</sup>, P. Webster<sup>1</sup>, L.N. Møller<sup>1</sup>, A. Malakauskas<sup>3</sup>, K. Noeckler<sup>4</sup>, E. Pozio<sup>5</sup>, P. Boireau<sup>6</sup>, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark<sup>1</sup>, Parasitological Institute, Slovak Academy of Science, Slovak Republic<sup>2</sup>, Lithuanian Veterinary Academy, Kaunas, Lithuania<sup>3</sup> Federal institute for Consumer Protection, Berlin, Germany<sup>4</sup> Istituto Superiore di Sanità Rome, Italy<sup>5</sup>, INRA/AFSSA Veterinary School Alfort, Paris, France<sup>6</sup>

The susceptibility, the serological response and the muscle distribution of three common European species of *Trichinella* (*T. spiralis*, *T. britovi* and *T. pseudospiralis*) were evaluated in equines. Thirty ponies were inoculated in groups of ten animals with 50,000 larvae of either *T. spiralis*, *T. britovi* or *T. pseudospiralis*. Five ponies from each group were killed at 5 and 10 week p.i. (wpi) and 15 different muscles/muscle groups were examined for larvae. At 5 wpi, infection was established in all three experimental groups with mean larval densities of 115, 10 and 18 larvae per gram (lpg), respectively. At 10 weeks p.i., mean larval densities were not significantly different: 112, 5 and 66 lpg. In ponies with high larval density infections, predilection sites of larvae included muscle groups with a relative high blood flow, i.e. tongue, masseter and diaphragm. *Trichinella*-specific antibody levels, measured by ELISA, increased during the first 3 weeks but decreased rapidly thereafter. Larvae released from horse muscle tissue were highly infective to mice. The high susceptibility, good persistency and uniform muscle distribution of all three species of *Trichinella* are new comparative findings that have relevance for the evaluation of epidemiology of *Trichinella* in horses and the meat inspection at the abattoir.

**8. High resolution analysis of genetic variability within *Trichinella* by non-isotopic single-strand conformation polymorphism and selective sequencing.** Robin B. Gasser<sup>1</sup>, Min Hu<sup>1</sup>, Youssef G. Abs El-Osta<sup>1</sup>, Dante S. Zarlenga<sup>2</sup>, Edoardo Pozio<sup>3</sup>, <sup>1</sup>Department of Veterinary Science, The University of Melbourne, Victoria 3030, Australia, <sup>2</sup>US Department of Agriculture, Beltsville, Maryland, USA, <sup>3</sup>Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy

A non-isotopic single-strand conformation polymorphism method was utilized to 'fingerprint' sequence variability in the expansion segment 5 (ES5) of domain IV and the D3 domain of nuclear ribosomal DNA within and/or among isolates and individual muscle (first-stage) larvae representing all of the currently recognized species/genotypes of *Trichinella*. Also, phylogenetic analyses of the D3 sequence data set, using different tree-building algorithms, established the relationships among all of them. These analyses showed strong support that the encapsulated species *T. spiralis* and *T. nelsoni* formed a group to the exclusion of the other encapsulated species *T. britovi* and its related genotypes *Trichinella* T8 and T9 and *T. murrelli*, and *T. nativa* and *Trichinella* T6, and strong support that *T. nativa* and *Trichinella* T6 grouped together. Also, these eight encapsulated members grouped to the exclusion of the non-encapsulated species *T. papuae* and *T. zimbabwensis* and the three representatives of *T. pseudospiralis* studied. The results showed that non-encapsulated species constitute a complex group which is distinct from the encapsulated species and supported the current proposal that encapsulated *Trichinella* group external to the non-encapsulated forms, in accordance with independent biological and biochemical data sets.

### 9. Identification of *Trichinella* isolates by means of 5S ribosomal DNA intergenic spacer region

**amplification and sequencing.** A. De Bruyne<sup>1</sup>, H. Yera<sup>1</sup>, F. Le Guerhier<sup>2</sup>, P. Boireau<sup>2</sup>, J. Dupouy-Camet<sup>1</sup>, *Trichinella* Reference Center<sup>1</sup>, Hôpital Cochin, 27 Fbrg St Jacques, 75014, Paris, UMR BIPAR<sup>2</sup>, Maisons-Alfort, France

A PCR based assay using a single pair of nucleotides amplifying 5S ribosomal DNA intergenic spacer regions was used to identify *Trichinella* isolates. We used the primers described in the study of Rombout et al. (J Clin Microbiol. 2001, 39:642-6) but, in which, isolates were identified after reverse line blot. In our study, amplified products were directly sequenced from both strands and compared to GenBank data. The isolates assayed were belonging to species usually found in Europe: *T. spiralis* (5 isolates), *T. nativa* (2 isolates), *T. britovi* (4 isolates), and *T. pseudospiralis* (one isolate). In addition, *T. nelsoni* (one isolate) and *T. murrelli* (two isolate) were tested. All isolates were part of the collection of one of us, they were kept frozen in alcohol or formalin and differed from those sequenced by Rombout et al. and submitted to GenBank. Amplified products of approximately 740 bp were obtained for 11 isolates. No amplified products were obtained in one *T. britovi*, two *T. murrelli* and one *T. nelsoni* isolates which were preserved in formalin. After one single amplification, we were able to identify *T. spiralis*, *T. britovi*, *T. nativa*. *T. spiralis* differed from *T. britovi* by 42 bp and from *T. nativa* by 41 bp; *T. britovi* differed from *T. nativa* by 22 bp. The *T. pseudospiralis* isolate was identified as *T. spiralis* suggesting an error in the identification of the tube kept in the collection. This simple assay, using a single pair of primers and sequencing, allows a rapid specific identification of *Trichinella* species. However, its routine use needs further evaluation.

**10. Genes encoding newborn larvae-specific, glutamic acid-rich proteins from both encapsulated and non-encapsulated *Trichinella* species.** D. S. Zarlenga, P. Boyd, M. B. Chute, D. Hill, J.R. Lichtenfels, US Department of Agriculture, ARS, ANRI, Beltsville, MD 20705, Beltsville, MD 20705, USA

One of the least characterized stages of *Trichinella* is the early L1 or newborn larval (NBL) stage, in which the parasite recognizes, penetrates and modulates reprogramming of the muscle cell. In light of evidence demonstrating strong protective characteristics of antigens derived from this stage, understanding this process is tantamount to effective abatement of infection. Herein, multiple clones were identified from a *T. spiralis* NBL cDNA expression library that reacted positively with swine infection serum and encode a family of glutamic acid-rich (GAR) proteins specifically transcribed in NBL. Sequence data predicted open reading frames (ORF) of 1497 bp (NBL1500) and 1716 bp (NBL1700) generating proteins of 498 aa and 571 aa, respectively. Both sequences consist of 38% glutamic acid and 16% serine residues, and differed only by the presence of a 219 bp fragment in NBL1700. PCR data indicate that more than one isoform can exist within a single worm and that isoform profiles can vary among individual worms within a population. Genotypic PCR profiles were separated into 2 groups; 1) *T. spiralis* and *T. nelsoni* which generated 2 equally intense fragments coincident with the NBL1500 and NBL1700 sequences and; 2) sylvatic genotypes, including all three non-encapsulated species, which presented single PCR fragments only. Comparing predicted ORFs from *T. spiralis*, *T. nelsoni* and the three non-encapsulated species indicated a lack of amino acid congruence primarily within the GAR region, with the most notable differences occurring between encapsulated and non-encapsulated species. Amino acid sequence data support the conclusion that the GAR proteins may be only partially conserved within the genus potentially linking variability to functionality.

**11. Molecular cloning and characterization of a novel antigen from *Trichinella spiralis*.** Zhu Xinping<sup>1</sup> Yang Jing<sup>1</sup> Yang Yaping<sup>1</sup> Ding Li<sup>1</sup> Huang Song<sup>1</sup> Zhou Lei<sup>1</sup> Boireau Pascal<sup>2</sup> Zhan Bin<sup>3</sup> Peter Hotez<sup>3</sup>, Department of Parasitology, School of Basic Medicine, Capital University of Medical Sciences, Beijing 100054, P. R. China<sup>1</sup>, INRA AFSSA ENVA UPVM, Maisons Alfort. France<sup>2</sup>, Department of Microbiology & Tropical Medicine, George Washington University, USA, Washington, DC, 20037

A cDNA library for adult *T. spiralis* was screened by immune antiserum and naturally infective serum from rabbits, 3 positive clones were identified. Sequence analysis showed that a novel antigenic gene Ts87 was found. It contained 1172 bp cDNA full length and encoded 347 amino acids. Recombinant protein Ts87 with molecular weight of 40KDa was produced in an E.Coli (PET-28) expression system and was purified with His-binding affinity chromatography. The recombinant protein was detected as positive reaction with patient sera infected with *T. spiralis*, infected swine sera and infected rabbit sera respectively by ELISA and Western-blot. The recombinant protein failed to react with the other parasite infected patient's sera. The anti-Ts87 immune serum and macrophage had a damage effect on new-born larvae in vitro, which appeared the effect of ADCC. Moreover, Ts87 recombinant protein induced a marked immune protection against *T. spiralis* in NIH mice. Immunocytolocalization demonstrated that Ts87 protein was found to be an excretory/secretory antigen and also rich on the cuticular surface of *T. spiralis* larvae. It is suggested that Ts87 recombinant protein would be taken as a new promising antigen that has potential both as an immunodiagnostic reagent and a vaccine antigen.

**12. Expression of the 30-mer peptide from *Trichinella spiralis* 43-kDa antigen on the surface of *E. coli* BL-21 and attenuated *Salmonella typhimurium* using the autotransporter MisL.** A.M. Castillo Alvarez<sup>1</sup>, N.Villegas-Sepulveda<sup>1</sup>, P. Ruiz-Olvera<sup>1</sup>, R. Fonseca-Liñán<sup>1</sup>, L.Yepez-Mulia<sup>2</sup>, C. González-Bonilla<sup>2</sup> and G. Ortega-Pierres<sup>1</sup>, Cinvestav IPN<sup>1</sup>, IMSS<sup>2</sup>. México. D.F. México

Mucosal immunization against *T. spiralis* has been successfully achieved by intranasal administration of a 30-mer peptide antigen with cholera toxin B (McGuire, C. et al. Infect. Immun. 7146, 2000). Although this can induce immunity other ways can be design to present this peptide for same purposes. Our group has reported the use of MisL, an autotransporter, to express foreign immunogenic epitopes on the surface of Gram negative bacteria ( Ruiz Perez, F. et al. Infect. Immun. 3611, 2002) which were able to induce an antibody response to the foreign epitope when given intragastrically to mice. In this work the MisL C-terminal translocator domain was used to display, residues 210 to 239. (RLEMYGSFLAKVMVVNMR-IWAVTDNTLQTT) of the 30-mer peptide from *T. spiralis* 43-kDa antigens on the surface of *E. coli* and *Salmonella enteric* serovar *typhimurium*. Plasmids containing the MisL domain were used to clone the peptide sequence from *Trichinella spiralis* fused to Flag tag and behind the *Escherichia coli* heat-labile enterotoxin B subunit signal peptide to assure periplasmic traffic. Expression of the fusion peptide was under the control of the anaerobiotically inducible nirB promoter. *E. coli* BL-21 and attenuated *Salmonella typhimurium* were transformed with these plasmids. Bacterial extract analysis by SDS-PAGE showed an over expression of a ~70 kDa protein which was confirmed by WB using MAb against Flag. Localization of the fusion peptide by IIF was shown in the bacterial surface. Current work is under way to test if the 30-mer fused peptide expressed in *S. typhimurium* is able to induce local immune responses to *T. spiralis*. This work was partially supported by a CONACyT (Mexico) grant No. G38523-M

**13. Immunoscreening of an adult worm cDNA Library of *Trichinella spiralis*: Cloning of a putative serine protease family in six variant types.** B.Q. Fu<sup>1,2</sup>, M.Y. Liu<sup>1</sup>, P. Boireau<sup>2</sup>, Y.L. Zhang<sup>1</sup>, X.P. Wu<sup>1</sup>, L.H. Yuan<sup>1</sup>, L.R. Li<sup>1</sup>, Q.J. Chen<sup>3</sup>, C.M.O. Kapel<sup>4</sup>, Changchun University of Agriculture and Animal Sciences, 175 Xian Road, Changchun, 130062, P.R.China<sup>1</sup>, UMR INRA-AFSSA-ENVA, 22, rue Pierre Curie, 94703, Maisons-Alfort, France<sup>2</sup>, Karolinska Institute, Stockholm, S-171 77, Sweden<sup>3</sup>, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Dyrlægevej 100, DK1870 Frederiksberg C, Denmark<sup>4</sup>

An adult cDNA library of *Trichinella spiralis* was screened using sera from a *T. spiralis* immunized pig. 33 positive clones were obtained after screening  $4.5 \times 10^5$  recombinant phages. 4 genes have been described before, while 16 new genes were found. Interestingly, the encoded amino acid sequences of 14 clones are highly homologous to each other, with comparable score (ClustalW) from 83 to 99, and encoding serine protease-like proteins. One of them, termed Zh68, contains a 1372 bp cDNA with an open reading frame of 1287 bp. The encoded polypeptide as a potential signal peptide sequence in the N-terminal end (1-18). The region of amino acid 37-277 composes the conserved domain of serine protease. His88, Asp142, Ser233 are predicted catalysis active sites. A glycosylation site at amino acid 78-81 (NGSQ) was predicted. There were six conserved cysteines likely mediating the formation of disulfide bridging. Blast search showed that this gene is novel with very low similarity to known *Trichinella* genes. Southern blots analysis indicated this gene belongs to a gene family with possible polymorphism. The gene was identified to be expressed in all of the stages. The recombinant antigen can induce immunity to the challenge infection. The recombinant proteins could be recognized by immune sera from swine and mice infected with *T. spiralis*.

**14. Cloning and identification of a cDNA encoding p46 kDa antigen from newborn larvae of *Trichinella spiralis*.** M.Y. Liu<sup>1</sup>, B.Q. Fu<sup>1,2</sup>, P. Boireau<sup>2</sup>, L.H. Yuan<sup>1</sup>, X.P. Wu<sup>1</sup>, Y.L. Zhang<sup>1</sup>, L.R.Li<sup>1</sup>, Q.J.Chen<sup>3</sup>, C.M.O. Kapel<sup>4</sup>, Changchun University of Agriculture and Animal Sciences, 175 Xian Road, Changchun, 130062, P.R.China<sup>1</sup>, UMR INRA-AFSSA-ENVA, 22, rue Pierre Curie, 94703, Maisons-Alfort, France<sup>2</sup>, Karolinska Institute, Stockholm, S-171 77, Sweden<sup>3</sup>, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Dyrlægevej 100, DK1870 Frederiksberg C, Denmark<sup>4</sup>

A specific clone (WN10) has been isolated from a cDNA library of newborn larvae (NBL) of *Trichinella spiralis* through immunoscreening with *T. spiralis* infected swine sera. Sequence analysis showed that this clone contained cDNA transcript of 1352 bp in length with a full open reading frame (ORF) of 1218 bp encoding a polypeptide of 406 amino acids with molecular weight of 45.9 kDa and isoelectric point of 5.43. A signal peptide and a potential N-glycosylation site have been identified, which indicated the protein is a secretory glycoprotein. There are two repeated regions in a 138 amino acids region with identity of 74%. A cystatin (cysteine protease inhibitor) motif in the C-terminal region of this protein has been identified. Further, the cystatin domain also shows certain degree of similarity to the two repeated regions but is different from any other nematode cystatins. The sequence of this cDNA has more than 98% identity to that of p46 kDa antigen of *T. spiralis* muscle larvae. PCR amplification with cDNAs from muscle larvae (ML), 3 day old adult (Ad3), 5 day old adult (Ad5), NBL libraries indicated that this gene is activated in all 4 stages. Fragments of WN 10 have been expressed in *E. coli* and Western blot revealed that the antigenic epitope of WN10 is in the two repeated regions, from amino acid 111 to 256 but not in the cystatin similar domain. Mice were immunized with the fusion protein of WN10, The data showed p46 kDa recombinant antigen could induce immune protection in mice against *Trichinella spiralis*.



**15. Cloning and analysis of a novel cDNA encoding a putative protein with FYVE zinc finger domain of *Trichinella spiralis*.** B.Q. Fu<sup>1,2</sup>, M.Y. Liu<sup>1</sup>, C.M.O. Kapel<sup>3</sup>, X.P. Meng<sup>1</sup>, Q. Lu<sup>1</sup>, X.P. Wu<sup>1</sup>, Q.J. Chen<sup>4</sup>, P. Boireau<sup>2</sup>, Changchun University of Agriculture and Animal Sciences, 175 Xian Road, Changchun, 130062, P.R.China<sup>1</sup>, UMR INRA-AFSSA-ENVA, 22, rue Pierre Curie, 94703, Maisons-Alfort, France<sup>2</sup>, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Dyrlægevej 100, DK1870 Frederiksberg C, Denmark<sup>3</sup>, Karolinska Institute, Stockholm, S-17177, Sweden<sup>4</sup>

A cDNA library of *Trichinella spiralis* adult worms 3 days post infection (AW3) was screened with a cDNA probe (T54) derived from a newborn larvae (NBL) subtracted cDNA library. A positive clone (pBS-T54) containing an insert of 1464 bp with a single open reading frame (ORF) of 1290 bp was targeted, which encodes a polypeptide of 429 amino acids of 49.9 kD and isoelectric point (IP) of 5.6. Motif scan analysis showed that the deduced protein had a leucine zipper motif in the N-terminal region and a FYVE zinc finger domain in the C-terminal region. Two potential ASN glycosylation sites at amino acids 12-15 and 103-106 were also found. Southern blot analysis indicated that a single copy was present in the genome of *Trichinella spiralis*. RT-PCR results revealed that this gene is expressed in all the developmental stages including muscle larvae, adult worms and newborn larvae stages. Recombinant fusion protein of T54 was expressed with pET 28 expression system in *E. coli* and rabbit immune sera were found to react with a single band migrating at approximately 55 kDa in crude worm extracts from muscle larvae, adults and NBL stages. The recombinant antigen was not recognised by serum of *Trichinella* infected pigs. \*The work was supported by grants of EU TRICHIPORSE QLRT-2000-01156, NSFC30328020, PRA BT0302 and AM1348NPP51.

**16. Cloning of a surprising DNase II family from *Trichinella spiralis*.** M.Y. Liu<sup>1</sup>, B.Q. Fu<sup>1,2</sup>, X.P. Wu<sup>1</sup>, C.M.O. Kapel<sup>3</sup>, Q. Lu<sup>1</sup>, C.Y. Li<sup>2</sup>, Q.J. Chen<sup>4</sup>, P. Boireau<sup>2</sup>, Changchun University of Agriculture and Animal Sciences, 175 Xian Road, Changchun, 130062, P.R. China<sup>1</sup>, UMR INRA-AFSSA-ENVA, 22, rue Pierre Curie, 94703, Maisons-Alfort, France<sup>2</sup>. Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Dyrlægevej 100, DK1870 Frederiksberg C, Denmark<sup>3</sup>. Karolinska Institute, Stockholm, S-17177, Sweden<sup>4</sup>

Seven genes putatively encoding deoxyribonuclease (DNase) II were cloned. Two similar genes belong to newborn larvae stage specific expression genes, and the other five similar ones expressed in both newborn and adult stages. One of the seven genes was expressed and the DNase II activity of the recombinant protein was found. The result was in contrast to the previous prediction that DNase II of *Trichinella* was probably without DNase II activity. Interestingly, by database searching a large number of new *Trichinella* DNase II (3 in full length and 11 in EST) were found, and which is much more than those in any other species (no more 4). The most dramatic finding was a unique conserved residue of histidine (H) found in N terminal end in the amino acid alignment of 66 available homologs from 35 species, and the downstream residue of histidine was either serine (S) or threonine (T). All DNase II homologs can be divided into two groups by the two conserved residues of HT or HS. The HT group is found in *Trichinella* and *Trichuris* while HS group is always found in other species. The position of this histidine was similar with activity histidine in serine protease between the first and second disulfide bridging cysteine, but it is in contrast to the previous reports that the activity histidine of DNase II is at the C-terminal end. In fact, the previous predicted histidine is not a really conserved residue, because this position is changed to lysine or serine in *Trichinella*. The potential functions of DNase II in *Trichinella* were discussed.

17. **Cloning of a gene encoding the cuticle collagen of *Trichinella spiralis*.** B.Q. Fu<sup>1,2</sup>, M.Y. Liu<sup>1</sup>, C.M.O. Kapel<sup>3</sup>, Q. Lu<sup>1</sup>, X.P. Wu<sup>1</sup>, C.Y. Li<sup>2</sup>, Q.J. Chen<sup>4</sup>, P. Boireau<sup>2</sup>, Changchun University of Agriculture and Animal Sciences, 175 Xian Road, Changchun, 130062, P.R. China<sup>1</sup>, UMR INRA-AFSSA-ENVA, 22, rue Pierre Curie, 94703, Maisons-Alfort, France<sup>2</sup>, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Dyrlægevej 100, DK1870 Frederiksberg C, Denmark<sup>3</sup>, Karolinska Institute, Stockholm, S-17177, Sweden<sup>4</sup>

A *Trichinella spiralis* 5-day-old adult worm (AW5) stage-specific cDNA fragment was identified in a subtractive cDNA library and was used as probe to screen the cDNA library to obtain the full length sequence. Sequence analysis showed that this cDNA was 1132 bp with one major open reading frame (ORF) of 1029 bp. The putative encoded polypeptide had a molecular weight of 35.1 kD, an isoelectric point of 4.87, a N-terminal signal peptide (Signal P v2.0) and a glycosylation site at 117-120 (SGYG). Blast search indicated that the N-terminal region (from AA 27 to 86) has high similarity to *Trichinella* cuticle collagen, the C-terminal region (AA153 to 328) of this protein is similar to collagen triple helix repeat domain. Southern blot analysis revealed only a single copy of this gene in *Trichinella spiralis* genome. The cDNA was cloned and expressed by pET 28 system in *E. coli* and the recombinant antigen was not recognised by the pig serum infected with *Trichinella spiralis*.

\*The work was supported by grants of EU TRICHIPORSE QLRT-2000-01156, NSFC30328020, PRA BT0302 and AM1348NPP51.

18. **Analysis of low complexity regions of the *Trichinella spiralis* genome for the resolution of polymorphic molecular markers.** G. La Rosa, G. Marucci, E. Pozio, Department of Infectious, Parasitic and Immunomediated Diseases. Istituto Superiore di Sanità. Viale Regina Elena 299, 00161 Rome, Italy

Polymorphic molecular markers have been widely used to study the population genetic structure and recently they were recognised as useful tools to conduct epidemiological studies. In *Trichinella*, the paucity of molecular markers makes difficult to study the population structure and consequently to approach relevant epidemiological and taxonomic aspects such as the transmission ways from wildlife to domestic and synanthropic animals, and the gene flow between parasite populations. In *T. spiralis*, only few genetic markers have been studied and only the expansion segment V locus was found polymorphic. Low complexity regions (LCR) are sequences with unusual composition, usually AT rich, characterised by dinucleotide runs or other short repeats. These regions may accumulate mutations during the DNA replication and, consequently, they represent an important source of polymorphisms. Over 1,500 sequences of *T. Spiralis* (average length 500 bp) were analysed, about 250 showed LCRs, and 100 of them fulfilled 2 conditions, i.e. they did not belong to putative ORF and suitable primer pairs could be designed for PCR amplification. The polymorphisms were searched in 9 *T. spiralis* isolates. Each sequence was amplified from a pool of larvae and to visualise the possible occurrence of co-amplification of multiple alleles electrophoretically indistinguishable, the PCR products were submitted to heteroduplex analysis. Thirty-four sequences showed a heteroduplex pattern in at least 1 isolate, suggesting the presence of a genetic polymorphism. The preliminary results show an unexpected high level of genetic variability in *T. spiralis*, both at the intra-isolate and at the inter-isolate levels. This variability can be exploited to conduct epidemiological and taxonomical studies. This work was funded by projects: #C3MO, Istituto Superiore di Sanità, and #3AAF, Italian Ministry of Health.

19. **Cloning and expression of a Glutathione-S-Transferase of *Trichinella spiralis*.** C. Bahuon, F. Le Guerhier, D. Le Rhun, E. Le Naour, P. Boireau, UMR BIPAR, 22 rue Pierre Curie, 94700, Maisons-Alfort, France

An extra-cellular Glutathione-S-Transferase (GST) purified from crude extracts of *Trichinella spiralis* muscular larvae has been previously described as an antigenic protein, and could thus be a candidate for diagnosis of trichinellosis in pigs (Rojas *et al*, 1997). Moreover, B- and T-cell epitopes from the GST of the parasite *Schistosoma mansoni* were used as a relevant anti-immunopathology and anti-infection vaccine against schistosomias (Lebens *et al*, 2003). The aim of this work was to clone and to express a GST of *T. spiralis*, to assess its antigenicity in order to develop an ELISA for an early diagnosis of pig trichinellosis. A *T. spiralis* New-Born Larvae (NBL) stage specific cDNA library was built in our laboratory. The Suppression Subtractive Hybridization allowed the identification of a sequence similar to the GST. A 5' 300bp part of this sequence was used to design a probe to screen an Adult/NBL cDNA expression library. Twelve clones were obtained which sequences were identical. This sequence was shown to be a full-length sequence with an open reading frame of 615bp, 205 amino acids, 24kDa and 47% of amino acids identity with the *Onchocerca volvulus* GST (pi class). The *T. spiralis* GST was expressed in pET102 vector in fusion with thioredoxin for solubility, using BL21 Star (DE3) *E. coli*, and the soluble thioredoxin-GST fusion protein was specifically purified with glutathione columns. The antigenicity of this recombinant protein is under investigation using Western-blot and ELISA with sera from conventional or specific-pathogen free pigs experimentally infected with *Trichinella*. Moreover, a 5'RACE reaction will be performed in order to get the sequence of the extra-cellular GST. This work was supported by the EU grant TRICHIPORSE QLRT-2000-01156.

20. **Identification of potential mediators of nurse cell transformation from *T. spiralis*.** D.B. Guiliano<sup>1</sup>, K. Gounaris<sup>1</sup>, and M.E. Selkirk<sup>1</sup>, <sup>1</sup>Imperial College London, Biological Sciences, South Kensington, London, U.K. SW7 2AY

Transformation of nurse cells by *T. spiralis* involves the cell cycle re-entry of the host cell nuclei, the loss of markers of terminal differentiation, and a subsequent blockade of nuclear division at G2/M. The molecular mechanisms by which the parasite initiates and maintains this transformation is unknown, however it is believed that parasite secreted (E/S) products interfere with the normal developmental program of the muscle and drive it down a novel pathway. Parasite E/S products have been analyzed in many studies and have been found to contain numerous enzyme activities including proteinases, kinases and nucleases. It has also been shown that the secreted products penetrate the nuclei of the invaded cells, indicating that they could have direct effects on processes such as DNA replication and gene transcription. The aim of this project is to identify novel proteins that are potentially involved in the myofibre transformation process. Publically available *T. spiralis* expressed sequence tag data has been searched for genes that are expressed during the nurse cell stage of the parasites development and have potential secretion signals, indicating they could be introduced into the host cell. Some of these proteins also contain other motifs such as potential nuclear localization signals that are not normally found on secreted proteins. These proteins are being expressed in either *E. coli* or *P. pastoris*, and anti-sera raised for western blot of E/S and immunohistochemical analysis of sectioned larvae and nurse cells. Many of the proteins found in this *in-silico* screen are novel, however several have homology to proteins such as macrophage migration inhibitory factor, saposins, granulins and porins. Assays are therefor currently being preformed to determine if these proteins are functional homologues of these gene families.

**21. Proteomic analysis of the excreted/secreted protein fraction of the *Trichinella spiralis* muscle larva.** M.W. Robinson, D. Gare and B. Connolly, Institute of Medical Sciences, School of Medical Sciences, University of Aberdeen, Scotland, UK, AB25 2ZD

Larval excretory/secretory (E/S) proteins have been implicated in the formation and maintenance of the parasite-host complex in *Trichinella* infections. ES proteins collected from both newborn and muscle L1 larvae elicit morphological and structural changes in primary rat myocytes in culture, although the identity of the specific proteins mediating these effects remains to be determined. Antigens sharing epitopes with *Trichinella* secreted proteins have been detected in isolated host nuclei and shown to co-localise to host nuclear chromatin complexes.

The identification and proof of the parasite origin of these antigens awaits data connecting the proteins with parasite-encoded gene sequences. Several activities have been identified in the ES fraction, including DNA binding, DNA endonuclease, protease and kinase activities and the recent application of proteomics has led to the identification of a 67kDa 5' nucleotidase and a putative serine protease. However, the majority of ES proteins have not been identified. Recently we have begun a proteomic analysis of *T. spiralis* ES proteins. Proteins have been separated by 2-dimensional electrophoresis and peptide mass fingerprint (PMF) data for >30 peptide spots has been generated by MALDI-TOF mass spectrometry. Interpretation of the PMF data has relied primarily of the interrogation of a custom-made *Trichinella*-EST database and the NemaGene Cluster database for *Trichinella*.

This has lead to the putative identification of ~17 proteins. Currently the identity of these proteins is being confirmed by post-source decay using Q-TOFII mass spectrometry.

**22. Identification and characterization of a stage-specific cDNA from adult worm of *Trichinella spiralis* that encodes a caveolin-1 protein.** R. Hernández-Bello<sup>1</sup>, R.M. Bermúdez-Cruz<sup>1</sup>, P. García-Reyna<sup>1</sup>, L. Mingyuan<sup>2</sup>, F. Le Guerhier<sup>3</sup>, P. Boireau<sup>3</sup>, G. Ortega-Pierres<sup>1</sup>, <sup>1</sup>Departamento de Genética y Biología Molecular, CINVESTAV, 07360, Mexico, D.F. <sup>2</sup>Changchun-University of Agriculture and Animal Sciences, Changchun, 130062, RP China, <sup>3</sup>UMR BIPAR, 94703 Maisons-Alfort, France

The identification and expression of specific genes of each different stage of *Trichinella spiralis* by subtractive hybridization allows the obtention of antigenic proteins which can be used in the detection and/or control of the trichinellosis. Also, the non-antigenic proteins can be analyzed considering their role in the establishment of the parasite during the course of the infection. A *T. spiralis* cDNA clone was obtained, confirmed as adult stage-specific by RT-PCR and used as a probe to screen a *T. spiralis*  $\lambda$ -ZAP cDNA library of 3 day-old adult worms. Three clones were selected and sequenced, the resulting consensus sequence showed matching on databases with caveolin-1 sequences. Analysis of the deduced-amino acid sequence (229 AA) showed 33% of identity to caveolin-1 of *C. elegans* and 28% of homology to caveolin-1 of mammals. The caveolin-1 of *T. spiralis* (Cav<sup>Ts</sup>) contains the caveolin signature motif, the putative membrane spanning, oligomerization and scaffolding domains. Although mammalian caveolin-1 has been reported to participate in several cellular processes, its biological function on parasites is not elucidated yet. The Cav<sup>Ts</sup> was expressed as a 43kDa fusion protein using the pET-102 vector. Preliminary western blots employing serum of experimentally infected pigs with *T. spiralis* did not shown antigenicity against this protein. The immunolocalization of Cav<sup>Ts</sup> is under investigation.

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**23. Studies on vertical transmission of *Trichinella* spp. in carnivores, pigs, and rodents.** P. Webster and C.M.O. Kapel, Danish Centre for Experimental Parasitology, Department of Veterinary Pathobiology, Royal Veterinary and Agricultural University, Denmark

Vertical transmission of *Trichinella spiralis* was evaluated in ferrets (n=21), foxes (n=11), pigs (n=12), guinea pigs (n=16), and mice (n=41). The placental barrier to be crossed by migratory *Trichinella* larvae varies structurally in different animal species. Ferrets and foxes have an endotheliochorial placenta structure, guinea pigs and mice a haemochorial, and pigs an epitheliochorial placenta. The non-encapsulating *Trichinella pseudospiralis* larvae have an extended muscle migration prior to entering a muscle cell. To evaluate if *T. pseudospiralis* was more likely to be transmitted to offspring, an additional group of foxes (n=11) infected with *T. pseudospiralis* was included. Two different dose levels were used for ferrets, pigs, guinea pigs, and mice. In pigs and guinea pigs, infection was given at different times of the gestation period. Vertical transmission, measured as recovery of muscle larvae in the offspring, was demonstrated in both ferrets groups, in all four guinea pig groups, and in the high dose mouse group, but not in any fox or pig groups.

**24. Intestinal establishment and reproduction of adult *Trichinella* spp. in mono and mixed infections in foxes (*Vulpes vulpes*).** P. Webster and C. M.O. Kapel, Danish Centre for Experimental Parasitology, Department of Veterinary Pathobiology, Royal Veterinary and Agricultural University, Denmark

Intestinal establishment and reproduction of adult *Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi* and *Trichinella pseudospiralis* were examined as mono or mixed infections in experimentally infected foxes. This is the first study of intestinal dynamics of *Trichinella* spp. in a carnivore model and the results suggest that the intestinal phase is relatively short since almost no worms were recovered 10 dpi. In mixed infection with equal doses of *T. nativa* and *T. spiralis*, molecular typing showed that 64% of the intestinal worms and 78% of the muscle larvae were *T. nativa*. Conversely, *T. spiralis* dominated in the mixed infections with *T. pseudospiralis*, constituting 66% of the intestinal worms and 94% of the muscle larvae. Although, the individual recoveries of intestinal worms were only up to 5.6% on day 1, and up to 1.5% on day 4 post infection, the muscle larvae establishment was comparable to other fox studies. Infectivity, measured as muscle larvae burden did not differ among the four species of *Trichinella*, which is in contrast to other models with mice, rats, pigs, or herbivores. Although some statistically significant differences in intestinal worm burdens were found, no single species were recovered in consistently higher numbers than the others.

25. **Congenital transmission of trichinellosis in the mice.** J. Cui, Z.Q. Wang, H.M. Hang, Department of Parasitology, Medical College, Zhengzhou University, Zhengzhou, 450052, P.R. China

Mice are the reservoir in domestic cycle of trichinellosis and have an important role in the transmission of *T. spiralis* infection, congenital transmission of trichinellosis was studied in BALB/c mice. Pregnant mice were each infected with 300 larvae at 5, 7, 15 and 17 days after mating. The moment of fertilization was subsequently calculated according to the date of birth for finding the gestation stage. New-born mice were examined by direct trichinoscopy and by peptic digestion of muscle. Out of 6 offspring born to the mother-mouse infected at 7 days after mating, two offspring were found to be infected, 7 and 24 larvae were recovered respectively. Other 7 female mice were first infected with *T. spiralis* and then gestated, only the offspring born to the mother-mice fertilized at 8 and 22 days after infection were found to be infected, the infection rate of offspring was 20% (2/10) and 25% (2/8) respectively, with a worm burdens ranging from 1-3 larvae per animal. All of larva recovered from the offspring were the unencysted larva. The cross-fostering in which one-day old young born to normal mother-mice were nursed by infected mothers for one month showed that no young were found to be infected. These findings showed that transplacental transmission of trichinellosis could occur in mice if female mice are infected during early pregnancy or mating in 1 month after infection, the larva transmitting from maternal-to-neonatal may be migrating one. The trans-mammary transmission of trichinellosis was not observed.

26. **The effect of progesterone in the *Trichinella spiralis* infection.** G.G. Nuñez, T. Gentile, S.N. Costantino and S.M. Venturiello, Chair of Immunology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, (1113) Argentina

In a previous work we demonstrated that during pregnancy there exists an increased helminthotoxic activity against *T. spiralis* newborn larvae (NBL), which is reflected in a low susceptibility to the parasite. Taking into account that the leukocytes are essential for larval death and that the progesterone ( $P_4$ ) is a pregnancy-associated hormone, the aim of this work was to assess the role of  $P_4$  in the NBL death. To this end, *in vitro* and *in vivo* studies were carried out. In cytotoxicity assays, peritoneal rat leukocytes were incubated with NBL and soluble  $P_4$  at different concentrations in the presence or absence of its antagonist mifepristone (RU 486, 200 ng/ml). In *in vivo* studies, two-month old Wistar rats were ovariectomised and injected (s.c.) with increasing concentrations of  $P_4$  during 21 days. At day 6 of the  $P_4$  treatment, animals were orally infected with 2000 muscle larvae (ML) per rat. At day 30 post-infection, parasite loads were determined by peptic digestion. Results showed the  $P_4$  was able to activate leukocytes which mediated NBL death *in vitro* (44.7±5.8% and 42.2±9.8% for 100 and 200 ng/ml of  $P_4$  vs control cells: 10.4±2.9%). This mortality was inhibited in the presence of RU 486 (3.7±1.8% and 10.1±3.7% for the same  $P_4$  concentrations). Parasite loads of  $P_4$ -treated animals was lower than that of the control group (896±490 ML/g vs 1679±367 ML/g;  $p < 0.01$ , Mann Whitney *U* test). From the results we can conclude that  $P_4$  can induce the activation of leukocyte population involved in NBL destruction (in an antibody-independent manner). This effect might explain the lower susceptibility of the animals to the parasite during pregnancy.

**27. Effects of fox, pig, sheep, and poultry bile and non-protein fraction of bile on the *in vitro* survival of domestic and sylvatic species of *Trichinella* sp.** G. Theodoropoulos<sup>1\*</sup>, M. Prokou<sup>1</sup>, V. Georgiadou<sup>1</sup>, M. Petrakos<sup>1</sup>, P. Webster<sup>2</sup>, C.M.O. Kapel<sup>2</sup>, Department of Anatomy and Physiology of Farm Animals, Faculty of Animal Science, Agricultural University of Athens, 75 Iera Odos, Votanikos, Athens 11855, Greece<sup>1</sup>, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Dyrlaagevej 100, DK-1870 Frederiksberg C, Denmark<sup>2</sup>

The *in vitro* differential effect of fox, pig, sheep, and poultry bile and corresponding non-protein fractions at various concentrations was examined on the motility of released muscle larvae of *T. spiralis*, *T. nativa* and *T. nelsoni*. In many cases the percentages of motile (live) larvae of the three *Trichinella* species cultured in the presence of the non-protein fraction of bile from the study animals were significantly higher ( $p < 0.001$ ) compared to their respective control cultures. In addition, the percentages of motile (live) larvae of all *Trichinella* species cultured in the presence of the non-protein fraction of bile at any concentration from all study animals, were significantly higher ( $p < 0.001$ ) compared to their respective cultures in the presence of raw bile. Not only the non-protein fraction of bile was different with the raw bile, but also the non-protein fraction with increased dilution showed a decrease in the percentages of motile (live) larvae while the opposite was true with the raw bile ( $p < 0.001$ ). These observations indicate that the non-protein fraction of bile prolongs the *in vitro* survival of larvae.

**28. Role of satellite cells in nurse cell formation.** Y. Takahashi, T. Boonmars, Z. Wu and I. Nagano, Gifu University Graduate School of Medicine, Department of Parasitology, 501-1194 Gifu Japan

*Trichinella spiralis* and *T. pseudospiralis* are intracellular parasites. It is long believed that they live in transformed muscle cells, named the nurse cell. Basically this thesis is correct, but needs some modifications. Immediately after entrance of newborn larvae of *T. spiralis*, muscle cells transform to the nurse cell losing its characteristic morphology but die through the apoptotic pathway. The satellite cell (myogenic stem cell) proliferates in response to the muscle cell damage. They do not differentiate to normal muscle cells but misdifferentiate to the nurse cells fusing with previous cells. The new nurse cells can survive for some time but eventually die. Again the satellite cell in the capsule supplies new nurse cells. Thus the nurse cell looks to survive continuously for years. In case of *T. pseudospiralis* infection, the infected muscle cells transform to the nurse cell. Satellite cells proliferate and misdifferentiate to nurse-cell like cells but do not fuse with the nurse cells.

**29. Clinical, hematological, biochemical and economic impacts of *Trichinella spiralis* infection in pigs.** M. Ribicich, H.R. Gamble, A. Rosa, A. Marquez, G. Mira, N. Cardillo, M.L Cattaneo and A. Franco, Parasitology and Parasitic Diseases, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Chorroarín 280, (C1427CWO) Ciudad de Buenos Aires, Argentina

The purpose of this work was to assess the clinical, hematological and biochemical responses of pigs experimentally inoculated with *Trichinella spiralis*. Groups of 3 pigs were inoculated per os with 100, 500, and 5000 *T. spiralis* muscle larvae. Clinical evaluation of disease in pigs included daily clinical examination, rectal temperature measurements and cardiac and respiration rates. Hematological studies included: hematocrit (%), hemoglobin (g/dl), and white cell, neutrophil, lymphocyte and eosinophil counts. Blood biochemistry included: bun (mg/dl), creatinine (mg/dl), AST (UI/l), ALT (UI/l), CPK (UI/l) and ALP (UI/l). One hundred days after inoculation, pigs were euthanized, and artificial digestion of 100 g of diaphragm from each pig was used to determine parasite burdens. No significant differences were observed in rectal temperature and in cardiac and respiration rates between inoculated animals and the control group ( $p > 0.05$ ). Significant differences were detected ( $p < 0.05$ ) in the values of hematocrit, % hemoglobin, white cell counts, eosinophils, neutrophils, and lymphocytes, as well as in the values of CK, ALP, AST and ALT. The variations observed were related to the number of *T. spiralis* larvae inoculated and varied with the number of days post-infection. Inoculated pigs showed significant differences ( $p < 0.05$ ) in weight gain when compared uninoculated controls. In infected pigs growth was reduced between 20% and 40%. The study of hematological parameters and enzymes, provides a better understanding of acute and chronic trichinellosis in pigs. Further, disease caused by *T. spiralis* results in a negative economic impact in production that could impact pig producers.

**30. *Rattus norvegicus* albino as a highly susceptible laboratory animal for maintenance of mongoose derived *Trichinella* larvae in Iran.** G.Mowlavi<sup>1</sup>, J.Massoud<sup>2</sup>, S.Soleymani Mohammadi<sup>3</sup>, K.Ashrafi<sup>4</sup>, S.Naddaf<sup>5</sup> I.Mobedi<sup>6</sup>, Ahvaz Health Research Station, School of Public Health, Tehran University of Medical Sciences, Postal Code, 14155-6446, Tehran, Iran

A variety of naturally infected reservoir hosts, such as different canids and wild boar, with *Trichinella* larvae have been reported from Iran. According to the biological and zoogeographical points of view, the parasite has been considered as *Trichinella nelsoni* in the Southwestern part of the country. Experimental infections of this nematode on laboratory animals have been successfully practiced with laboratory mice so far, while *Rattus norvegicus* albino has not shown similar susceptibility to this species of *Trichinella*. *Trichinella nelsoni* is found throughout the equatorial Africa and Southern Europe. It has no tolerance to freezing, a relatively high tolerance to heat, and has a low infectivity for rats. Previous studies show the susceptibility of laboratory mice to infection with *Trichinella* larvae originated from Southwestern part of Iran. In the present study, we tried to challenge *Trichinella* larvae recovered from naturally infected *Herpestes auropunctatus* to both laboratory rats and mice. In contrast with previous observations in this area, our findings showed a different susceptibility of laboratory mice to the infection, while *Rattus norvegicus* albino was highly susceptible to this mongoose derived *Trichinella* larvae. Our data suggest that the wild life of Southwestern Iran might be infected with more than one species of *Trichinella*, so exact identification of the members of the genus in this region remains to be clarified by molecular studies. This is the first report of natural infection of *H. auropunctatus* with *Trichinella* spp. in Iran.



**31. Evaluation of the infectivity of *Trichinella papuae* and *Trichinella zimbabwensis* for equatorial freshwater fishes.** E. Pozio and G. La Rosa, Istituto Superiore di Sanità, Rome, Italy

The discovery of *Trichinella* species infecting poikilotherm vertebrates has open new scenarios in the epidemiology of this parasite group. The aim of the present work was to investigate the infectivity of the 2 non-encapsulated species of *Trichinella* infecting both mammals and reptiles, namely *Trichinella papuae* and *Trichinella zimbabwensis*, for equatorial freshwater carnivore fishes. We selected piranha belonging to the species *Serrasalmus nattererii* and *Serrasalmus rombeus*, because they live at a temperature of water ranging from 25 to 32°C, which is the same temperature range at which *T. papuae* and *T. zimbabwensis* are able to complete their life cycle in reptiles. Fishes were inoculated per os with 1,000 larvae each. Four fishes received *T. papuae* and 4 fishes *T. zimbabwensis*. Mice were infected with 500 larvae of the two species from the same batches as controls. Six days post infection (p.i.), one fish for each *Trichinella* species was sacrificed, and worms were searched in the intestine, celomatic cavity and in the muscles under a dissection microscopy. The other fishes were sacrificed 60 days p.i. and worms were searched in the intestine and celomatic cavity under a dissection microscopy and in the muscles by artificial digestion. No larva or adult worm were detected in any organ or tissue 6 and 60 days p.i.; whereas, control mice were infected 60 days p.i. The lack of infectivity of *T. papuae* and *T. zimbabwensis* for fishes suggests that the entozoic habitat of this low vertebrate class does not represent a suitable environment for these two *Trichinella* species. These results allow us to exclude that freshwater fishes, one of the food resources for crocodiles, caimans and alligators, can play a role in the epidemiology of the known species of the genus *Trichinella*. Work funded by the project of the Istituto Superiore di Sanità, contract C3MO.

**32. Infectivity of *Trichinella* spp. in red foxes.** C.M.O Kapel<sup>1</sup>, P. Webster<sup>1</sup> and A. Malakauskas<sup>2</sup>, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark<sup>1</sup>, Lithuanian Veterinary Academy, Kaunas, Lithuania<sup>2</sup>

Carnivores are considered to be universal hosts of most *Trichinella* species, and although intensively studied in mice and rats, comparative studies in carnivores are rare. In the present study, the infectivity, persistence, and antibody response of nine well-defined genotypes of *Trichinella* was compared in 108 red foxes (*Vulpes vulpes*, 10 wk of age). Each fox was inoculated with 10.000 larvae and blood serum was collected prior to inoculation and at necropsy (10, 20 and 40 wks pi). Ten different muscle samples from each fox were examined by digestion for an estimation of the total larval burden. All genotypes had high infectivity, persisted until the end of experiment (40 wpi) and larvae collected from the fox tissue were all highly infective to mice. Accordingly, all foxes showed strong antibody responses. It was evident that both encapsulating and non-encapsulating *Trichinella* had comparable and high infectivity and good persistency in the foxes. This contrasts findings from rats and pigs where only the domestic species *T. spiralis* is highly infective and persistent. These results establish that foxes are suitable indicator animals for epidemiological monitoring and appropriate hosts for comparative studies on biological characteristics of both sylvatic and domestic genotypes of *Trichinella*.

**33. Fusion and differentiation in mammalian skeletal muscle cells that express *Trichinella spiralis* p43.** D. P. Jamer, X. Cheng and D. Kwak, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, 99164-7040

The ability of a 43 kDa stichocyte protein from *Trichinella spiralis* (Tsp43) to interfere with mammalian skeletal muscle gene expression was investigated. A MYC-tagged Tsp43 construct was expressed as a recombinant protein in C2C12 myoblasts. Transfection with low amounts of expression plasmid (0.25  $\mu\text{g ml}^{-1}$ ) was required for successful expression of the protein. Moderate toxic effects on transfected myoblasts was attributed to transfection with this construct. In addition, ectopic GFP expression was suppressed in myoblasts cotransfected with the Tsp43 construct. These effects may reflect similarities of Tsp43 to DNase II. *Tsp43* gene transfected myoblasts expressed myosin heavy chain when cultured under differentiation conditions, although the toxic and suppressive effects of the *Tsp43* gene introduced some reservations on interpretation of these results. The general DNase inhibitor, aurintricarboxylic acid (ATA), reduced the toxic and suppressive effects of *Tsp43*. Transfected myoblasts cultured in ATA underwent fusion and differentiation. These results support that Tsp43 did not inhibit muscle differentiation by interference with helix-loop-helix interactions among muscle differentiation factors, a possibility suggested from theoretical considerations. Collectively, the results support that Tsp43 has a role in the *T. spiralis* life cycle that is distinct from repressing muscle gene expression during the muscle phase of infection. While the function of Tsp43 as a DNase is under debate, the effects of ATA on transfected muscle cells are consistent with this possibility.

**34. Increased expression of a new antioxidant enzyme in the nurse cell during *Trichinella britovi* infection as revealed by "in situ" hybridisation.** S. Piaggi<sup>1</sup>, A. Salvetti<sup>2</sup>, L. Rossi<sup>2</sup>, M. Saviozzi<sup>1</sup>, V. Gremigni<sup>2</sup>, A. Casini<sup>1</sup> and F. Bruschi<sup>1</sup>, Depts of <sup>1</sup>Experimental Pathology, M.B.I.E. and of <sup>2</sup>Human morphology and Applied biology, Università di Pisa, Italy

Ascorbic acid (AA) is an important factor of defence against oxidative stress. AA is maintained in the reduced functional form by a number of enzymes including a new DHA reductase (DHAR), purified from rat liver cytosol, human red blood cells and cloned from a rat liver and a radiation resistant mouse lymphoma cell line. Among the different aspects of the host-parasite relations at muscle level in trichinellosis, the role of anti-oxidant system have rarely been studied to date. To better elucidate these aspects, we investigate the ability of *Trichinella britovi* and of the muscle cell infected to produce the DHAR. Biochemical data, immunoblot analysis and immunohistochemical studies suggested the absence of this protein within the parasites while we observed an increased amount of DHAR in the nurse cell (NC) compared to the surrounding muscle fibres. To evaluate the expression level of DHAR we performed the "in situ" hybridisation at different infection times. Diaphragms from mice, experimentally infected per os with 500 L<sub>1</sub> larvae, at 30 and 70 days of infection and control mice were isolated and processed for the "in situ" hybridisation. The results show that no relevant amount of DHAR mRNA is present in the parasite but the NC results strongly stained with respect to the surrounding muscle cells at infection times evaluated. The results will be discussed in the light of host-parasite relations.

**35. Detection of syndecan-1 in muscle cells infected with *Trichinella spiralis*.** Daniel P. Beiting,<sup>\*1</sup> Pyong Woo Park,<sup>2</sup> Judith A. Appleton<sup>1</sup>, <sup>1</sup>James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA, <sup>2</sup>Department of Medicine and Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA

Syndecans are a family of cell surface, transmembrane proteoglycans found on all adherent cells. Members of the syndecan family are comprised of a core protein modified by numerous, highly sulfonated heparan sulfate chains that mediate interactions with extracellular matrix, growth factors, cytokines and chemokines. One member of this family, syndecan-1, is expressed on the surface of epithelial cells, endothelial cells, plasma cells, and immature skeletal muscle cells. In this study we show that mature muscle cells infected with *Trichinella spiralis* are induced to express syndecan-1. Immunohistochemical analysis of nurse cell syndecan-1 demonstrated cytoplasmic and extracellular distribution of the protein, rather than conventional, cell surface localization. To examine the role of syndecan-1 in the intracellular habitat of *T. spiralis*, we infected wild-type and syndecan-1 deficient mice by intravenous injection of *T. spiralis* newborn larvae. Nurse cells developed to maturity, and the inflammatory response to muscle infection was largely unchanged in the absence of syndecan-1. In addition to syndecan-1, we also detected perlecan, a related proteoglycan, and heparan sulfate associated with the nurse cells in both wild-type and syndecan-1 deficient mice. This suggests that other heparan sulfate-bearing proteoglycans may compensate for a loss of syndecan-1.

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**36. Over expression of Hsp60 in tongue and diaphragm and Hsp70 in small intestine of rats infected with *Trichinella spiralis*.** Ma. Guadalupe Basurto Frausto, Berenice Luna Sánchez, Alejandra Moreno García, Olga Y. Barbosa-Cisneros, Sergio H. Sánchez-Rodríguez, Unidad Académica de Biología Experimental, Departamento de Biología Celular y Microbiología, Universidad Autónoma de Zacatecas, Apartado Postal 12, Guadalupe, Zacatecas, C.P. 98600

Trichinellosis is a parasitic disease caused by *Trichinella spiralis*, that infect the muscle of all mammals. The Heat shock proteins (Hsp) are expressed in all organisms and are induced by a number of stress agents such as parasitic infection. Objective: We determined the expression of the Hsp25, 27, 60, 70 and 90 during the intestinal and muscular stage of *T. spiralis* in Long Evans rats. Material and Methods: We obtained, of the infected rats with *T. spiralis*, serum, small intestine, tongue, diaphragm, masseter and leg muscle. We characterised the antigenic determinants and Hsp in tissue by SDS-PAGE, Western blot and immunofluorescence. Results: We found that *T. spiralis* implants in intestine at the fifth day which is correlated with the detection of antigenic determinants at the same time. The tissue of the healthy rat in leg, masseter muscle, tongue and diaphragm express in constitutive way the Hsp 25 and 70. In infected tissue expression increased in tongue and diaphragm. The Hsp 60 is not expressed in healthy tissue and in infected tissue is localized in tongue and diaphragm. Hsp 90 is only expressed in healthy rat diaphragm and is not express in infected rats. Infected intestinal tissue overexpressed Hsp 70.

**37. The virulence of *T. spiralis* is due of its adaptation mechanisms conferred by the heat shock proteins 25 and 90.** Berenice Luna Sánchez, Ma. Guadalupe Basurto Frausto, Alejandra Moreno García, Olga Y. Barbosa Cisneros, Sergio H. Sánchez-Rodríguez, Unidad Académica de Biología Experimental, Departamento de Biología Celular y Microbiología, Universidad Autónoma de Zacatecas, Apartado Postal 12, Guadalupe, Zacatecas, C.P. 98600

Summary: Heat shock proteins (Hsp) are expressed in all organisms and are over expressed due to stress caused by different agents such as the infection by *T spiralis*. Trichinellosis is a parasitic disease caused by *Trichinella spiralis*, that infects the muscles of all mammalian. Objective: We determined the expression of the Hsp25, 27, 60, 70 and 90, when the *T. spiralis* nematode is exposed to different temperatures and low pH. Material and Methods: *T. spiralis* was obtained of Long Evans rats by artificial digestion and submitted to different temperatures (4, 21, 40 or 60°C) and pH of 4. Larvae were processed by indirect immunofluorescence, PAGE-SDS and Western blot. Results: When analyzing the expression of Hsp proteins 25, 27, 60, 70 and 90 in stressed nematodes, we detected only Hsp 25 and 90. The quantification analyses by Western blot showed the expression of Hsp 25 and 90 with an increase in the expression of Hsp90 with heat. When we applied low temperatures Hsp 25 increased. Conclusion: *T. spiralis* survives sudden temperature changes due to support by the heat shock proteins 25 and 90.

**38. Distribution of *Trichinella britovi* larvae in muscles from experimentally infected foxes (*Vulpes vulpes*).** A. Marinculic<sup>1</sup>, R. Beck<sup>1</sup>, D. Mihelić E<sup>2</sup>. Pozio<sup>3</sup>, K. Sever<sup>4</sup>, J. Ristić<sup>1</sup>, Department of Parasitology<sup>1</sup>, Department of Anatomy, Histology and Embryology<sup>2</sup>, Chair for Game Biology, Pathology and Breeding<sup>4</sup>, Veterinary Faculty, University of Zagreb, Heinzelova 55, 10 000 Zagreb, Croatia; Laboratory of Parasitology<sup>3</sup>, Laboratory of Parasitology, Istituto Superiore di Sanità, viale Regina Elena 299, 00161, Rome, Italy

The aim of the study was to describe the results of the analysis of distribution of muscle larvae in red foxes. Three foxes, two months old, were inoculated with 1000 larvae of *T. britovi* (ISS 2). 45 days after inoculation foxes were sacrificed and tissue samples from 25 selected muscles from both sides were examined. In order to define the real distribution two samples from each muscle were taken. A larval distribution was determined by comparing larval counts in one gram of tissue and larval counts in rest of the muscle. Both samples were artificially digested and the number of larvae was defined. Comparison of the number of larvae from each muscle of the left and right side of the body revealed a difference in larval distribution in almost half of examined muscles. The comparison revealed unequal larvae distribution.

**39. Effect of extract *Usnea florida* lichen on the implantacion and fecundity of adult female Balb/c mouse infected with *Trichinella spiralis*.** E. Jiménez-Cardoso<sup>1</sup>, M.L. Caballero-García<sup>1</sup>, R. Mateo-Gonzales, R.M. Chapa-Ruiz<sup>2</sup>, E. Angeles-Angiano<sup>2</sup>, <sup>1</sup>Parasitology Research Laboratory, Hospital Infantil de Mexico Federico Gómez, <sup>2</sup>Inmunoparasitology Laboratory, E.N.C. B. IPN, <sup>3</sup>Pharmacology Laboratory, UNAM Mexico City

The aim of the present investigation, was to determine the effect of the extract *Usnea florida* on the implantation, viability and fecundity of the female BALB / c mouse with *T. spiralis*. We made groups with 4 mouse each/one. Group 1; control animals non infected; group 2, positive controls infected animals with *T. spiralis* and no treatment with *Usnea florida*; group 3 infected animals with *T. spiralis* that were treated with 20 mg/kg of albendazol; group 4, animals infected with the *T. spiralis* that received 700µg/ml of extract of *Usnea florida* dissolved in dimetil sulfoxido at 4% and finally group 5, with animals infected with *T. spiralis* that received 100 µl of dimetil sulfoxido at 4%. The blood samples obtained days 0, 3 and 5 post-treatment. The Polymerase Chain Reaction (PCR) was development with 700 ng of DNA and 500 ng of primers 500 ng of primers pPRA (Dick et al J. Parasitology 1992). In the day 7 post-treatment the animals were sacrificed, and the thin intestine female worms were obtained. The last ones were cultivated on RPMI for 48 hours and the newly larva born were count, by microscopy with lens of 10X and 40X. The reduction of the extract *Usnea florida* on the implantation of the female of *T. spiralis* was of 67%. The relationship to the fecundity of female worms was observed with a reduction of 43%. The technique of PCR detected the presence of ADN of the parasite in mouse infected with *T. spiralis*. These results suggested that the *Usnea florida* can be used as an antihelmintic product in newly born larvae.

**40. IgE enhances clearance of *Trichinella spiralis* and regulates mast cell responses in mice.** M.F. Gurish<sup>1</sup>, P. Bryce<sup>2</sup>, H. Tao<sup>1</sup>, A.B. Kisselgof<sup>2</sup>, E. Thornton<sup>3</sup>, H.R.P. Miller<sup>3</sup>, K.F. Austen<sup>1</sup>, D. Friend<sup>1</sup> and H. C. Oettgen<sup>2</sup>. Departments of Medicine, Brigham and Women's Hospital<sup>1</sup>, Children's Hospital<sup>2</sup>, and Harvard Medical School, Boston, Massachusetts, and Royal (Dick) School of Veterinary Studies<sup>3</sup>, University of Edinburgh, Easter Bush, Scotland

*Trichinella spiralis* infection elicits a vigorous IgE response, a pronounced mastocytosis in the intestine and spleen and an eosinophilia in the blood, intestine and around encysting larvae in skeletal muscle. Since IgE both activates mast cells (MC) and promotes their survival in culture, we examined role of IgE in MC and eosinophil responses and in parasite elimination in *T. spiralis*-infected mice. During primary infection, wild-type but not IgE-null (IgE<sup>-/-</sup>) BALB/c mice mounted a strong IgE response peaking 14 days after infection. The splenic mastocytosis was reduced in IgE<sup>-/-</sup> mice while the jejunal mastocytosis and the eosinophilia in the blood, jejunum and muscle were normal. Despite the normal MC response in the small intestine, serum levels of mouse MC protease-1 were lower in parasite-infected IgE<sup>-/-</sup> animals and these animals were slower to eliminate the adult worms. The number of *T. spiralis* larvae present in the skeletal muscle of IgE<sup>-/-</sup> mice 28 days after primary infection was about twice that in BALB/c controls, and the fraction of larvae that was necrotic was reduced in the IgE-deficient animals as seen also in CCR3<sup>-/-</sup> which lack the eosinophils around cysts. An intense deposition of IgE in and around the muscle larvae was observed in wild-type but not in IgE null mice. Since CCR3 deficient mice also have more larvae encysted in the muscle, we conclude that IgE promotes parasite expulsion from the gut following *T. spiralis* infection and participates with the eosinophil in elimination of the larval stages of the parasite. Furthermore, our observations support a role for IgE in the regulation of MC homeostasis in vivo.

41. **Chemokine changes in mast cells stimulated by TSL-1 antigens.** S. Lugo- Hernández<sup>1,2</sup>, E. García-Zepeda<sup>2</sup>, M. Ramírez<sup>2</sup>, G. Ortega-Pierres<sup>3</sup>, N. Arizmendi-Puga<sup>1</sup>, and L. Yépez-Mulia<sup>1</sup>. UIMEIP-IMSS, Mexico,<sup>1</sup> Instituto de Investigaciones Biomédicas, UNAM, México, <sup>2</sup>CINVESTAV-IPN, México<sup>3</sup>

We have demonstrated that the activation of mast cells (MC) by *T. spiralis* antigens (TSL-1 antigens) by an IgE independent mechanism, induce the release of histamine and an increase in their mRNA and protein levels for IL-4 and TNF. It was of interest to determine if MC stimulated by TSL-1 antigens can also be a source of other pro-inflammatory molecules such as chemokines. We focused our analysis on MCP-1/CCL2, a CC chemokine that has been detected in *T. spiralis* infected animals. Therefore, in this work we studied by RT-PCR the mRNA changes for CCL2 in HRMC mast cell line stimulated with 50 and 200 ng/ml TSL-1 for 8 h. An increase in mRNA expression for CCL2 was observed at 200 ng/ml TSL-1 that was twice the observed in the control. To confirm that the increase in mRNA levels of CCL2 correlates with an increase in its protein expression, we determined by ELISA the release of this chemokine. In fact, activated HRMCs released CCL2 and the maximum release (5000 pg/ml) was observed when 50 ng/ml TSL-1 was used. Our data suggest that MC may be an important source for CCL2 and participate in the regulation of the inflammatory process observed during the intestinal infection. We are currently investigating the significance of these findings by assessing specific CCL2-mediated functions on HRMC cells.

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42. **TSL-1 antigens and substance P activate mast cells in a similar manner.** N. Arizmendi<sup>1</sup>, J.A. Enciso<sup>1</sup>, G. Ortega Pierres<sup>2</sup>, D. Befus<sup>3</sup> and L. Yépez-Mulia<sup>1</sup>, UIMEIP-IMSS, Mexico,<sup>1</sup> CINVESTAV-IPN, México<sup>2</sup>, Pulmonary Research Group, University of Alberta, Canada<sup>3</sup>

Degranulation of mast cells (MC) by an IgE independent stimulation occurs by exposure to different substances such as substance P, compound 48/80, polylysine and human neutrophil defensins. In this process, G proteins are involved. We have also showed that TSL-1 antigens from *T. spiralis* induce histamine secretion from unsensitized peritoneal mast cells (PMC). Thus, we evaluate if degranulation induced by TSL-1 antigens shares features to substance P induced secretion. For this, PMC were incubated with 30 ng/ml of TSL-1 or 10<sup>-5</sup> M substance P and time course histamine release was determined by a fluorimetric assay. PMCs from *N. brasiliensis* infected rats were incubated with the homologous antigen and included as an IgE dependent histamine release control. In addition, inhibition of histamine release from stimulated PMC pretreated with *B. pertussis* toxin (1-100 ng/ml) or neuraminidase V (0.01 – 0.1U/ml) was also determined. The results showed that histamine secretion induced by TSL-1 and substance P was completed at 10 and 15 sec respectively. However, histamine release by an IgE-allergen dependent pathway was submaximal at 15 sec. In addition, histamine release induced by TSL-1 antigens or substance P was inhibited by *B. pertussis* toxin and neuraminidase V. We conclude that, in fact, histamine release induced by TSL-1 antigens has features similar to substance P induced histamine secretion. The signalling pathway involved in the activation of MC by TSL-1 antigens is under analysis.

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**43. Expression of *Trichinella spiralis* DNA vaccine in mammalian cells.** J. Cui<sup>1</sup>, Z.Q. Wang<sup>1</sup>, H.M. Han<sup>1</sup>, R.L. Li<sup>2</sup>, Dept. of Parasitol., Medical College, Zhengzhou University, Zhengzhou<sup>1</sup>, Dept. of Parasitol., Tongji Medical College, Huazhong University of Science and Technology, Wuhan<sup>2</sup>, China

DNA vaccine (pcDNA3-TspE1) encoding a 31 kDa antigen of *T. spiralis* was transfected into CHO cells with Lipofectamine 2000. The positive cell clones were screened by the selective antibiotic G418. The products expressed were identified by RT-PCR, IFAT, SDS-PAGE and Western blot. The results of RT-PCR amplification showed that there was one band with 876bp in CHO cells transfected with pcDNA3-TspE1 and no any bands in CHO cells transfected with only pcDNA3. The IFAT demonstrated that the pcDNA3-TspE1 transfected CHO cells were reacted with sera from mice immunized with the recombinant fusion protein 31 kDa and infected with *T. spiralis*. The pcDNA3 transfected and no-transfected CHO cells are negative reaction. SDS-PAGE showed that there was one band with 31 kDa in culture supernatant of CHO cells transfected with pcDNA3-TspE1. Western blot confirmed that the band with 31 kDa could be recognized by sera from mice immunized with recombinant fusion protein, rabbits immunized with *T. spiralis* larval soluble antigens, mice infected with *T. spiralis* and from patients with trichinellosis. We conclude that mammalian CHO cells were transfected by pcDNA3-TspE1. The TspE1 gene of *T. spiralis* was expressed in the transfected CHO cells. The proteins expressed could be secreted into cell culture supernatants and had the antigenicity of *T. spiralis*.

**44. Expression of *Trichinella spiralis* DNA vaccine in skin and muscle of BALB/c mice.** J. Cui<sup>1</sup>, Z.Q. Wang<sup>1</sup>, H.W. Zhang<sup>1</sup>, B.L. Xu<sup>2</sup>, Department of Parasitology, Medical College, Zhengzhou University, Zhengzhou 450052<sup>1</sup>, Health and Anti-epidemic Center of Henan Province, Zhengzhou 450003<sup>2</sup>, P.R. China

DNA vaccine (pcDNA3-TspE1) contained the gene encoding a 31 kDa antigen of *T. spiralis* was constructed. BALB/c mice were immunized with plasmid DNA vaccine by intramuscular injection and gene-gun delivery. The transcriptional activity of the pcDNA3-TspE1 in skin and muscles at the site of inoculation was investigated by RT-PCR. The expression of TspE1 gene in skin and muscles was detected by immunohistochemistry and IFA, respectively. RT-PCR products were obtained only from the skin and muscle samples of mice inoculated with pcDNA3-TspE1, but not in those mice inoculated with only the empty plasmid pcDNA3. The results of immunohistochemical staining demonstrated that the specific brown round particles were seen among cells and in the cytoplasm of epidermis cells in the mice immunized with pcDNA3-TspE1, but not in the mice immunized with pcDNA3. The results of IFA showed that the frozen section of muscle at inoculation site with pcDNA3-TspE1 was reacted with sera from mice immunized with recombinant fusion protein 31 kDa antigen or infected with *T. spiralis*. These results indicated that pcDNA3-TspE1 was successfully transcribed and expressed in skin and muscles at the site of inoculation of mice. Thus, the plasmid encoding 31 kDa antigen may be of value for further development of DNA vaccine against swine trichinellosis.

**45. Vaccination of mice with DNA vaccine induces immune response and protection against *T. spiralis* infection.** Z.Q. Wang<sup>1</sup>, J. Cui<sup>1</sup>, H.M. Han<sup>1</sup>, H.Y. Wei<sup>1</sup>, H.W. Zhang<sup>1</sup>, R.L. Li<sup>2</sup>. Department of Parasitology, Medical College, Zhengzhou University, Zhengzhou 450000<sup>1</sup>, Department of Parasitology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, P.R. China

IFA showed that sera from mice vaccinated with pcDNA3-TspE1 by intramuscular injection or gene-gun delivery reacted with *T. spiralis* larval antigen, the specific fluorescence was mainly situated at the cuticle of larvae. Western blot demonstrated that sera from mice immunized with pcDNA3-TspE1 only reacted with a 31kDa antigen component of *T. spiralis* larvae. Serum anti-*Trichinella* antibody was detected 2 wk after the last vaccination, then increased, and lasted at least for 4 wk by ( at the end of experiment). when stimulated with the recombinant fusion protein there was a higher proliferation of spleen cells from mice vaccinated with pcDNA3-TspE1. Levels of CD4<sup>+</sup> cells in peripheral blood of immunized mice increased obviously, the levels of CD8<sup>+</sup> cells elevated slightly, the ratio of CD4<sup>+</sup>/ CD8<sup>+</sup> cells increased evidently. After challenge infection with *T. spiralis*, the levels of CD4<sup>+</sup> cells decreased and levels of CD8<sup>+</sup> cells increased in mice immunized with DNA vaccine. Two and four weeks after the last immunization, the immunized mice were subsequently infected with *T. spiralis* larvae, exhibited from 36.5% to 37.37% of reduction in muscle larval recovery. DNA vaccine induced cellular and humoral immune response, and partial immune protection against challenge infection with *T. spiralis* larvae.

**46. Influence of adjuvant formulation on induced host protection in a mouse vaccination model against *Trichinella spiralis*.** S. Deville<sup>1,2</sup>, A. de Pooter<sup>1,2</sup>, V. Lainé-Prade<sup>1</sup>, M. Cote<sup>1</sup>, S. Ascarateil<sup>2</sup>, J. Aucouturier<sup>1,2</sup>, P. Boireau<sup>1</sup>, I. Vallée<sup>1</sup>. <sup>1</sup>UMR BIPAR, Maisons-Alfort, France, <sup>2</sup>SEPPIC, Paris, France

Vaccination of pigs could be a good alternative to prevent the risk of zoonosis. In order to develop an efficient and safe vaccine, the choice of the adjuvant is an important issue. We had already selected some efficient adjuvant formulations in mice in previous work (Parasite 2001, 8: S126-S132) nevertheless the correlation between the immune response and potential protection was not characterized. Indeed, vaccination tests were performed in mice using different adjuvant formulations and *Trichinella* antigens, then animals were challenged and their immune response was analyzed. Specific IgG1 and IgG2a were analyzed for evaluation of respectively the humoral and cellular response to the vaccine as well as IgE (total and specific). Cytokine production (INF $\gamma$  and IL12) was also tested after vaccination and challenge. Adjuvant safety was checked by the assessment of the local reactions at the injection site. Two Montanide<sup>TM</sup> ISA water in oil emulsions based on mineral oil (ISA 70) or non mineral oil (ISA 775), nanoparticles Montanide<sup>TM</sup> IMS and polymeric adjuvant were tested. Aluminium hydroxide adjuvant was used as a reference adjuvant. The results clearly show differences in antibody responses induced by adjuvants and differences in protection level. This experiment demonstrates the necessity to use an adjuvant to obtain a specific IgG1 or IgG2a and IgE response directed against the total soluble extract of *T. spiralis*. All the formulations enhanced a humoral immune response while the ISA70 and the polymeric adjuvant were able to induce a strong cellular response. ISA70 induced the highest level of protection with a decrease of 84.5% in parasitic burden. Emulsions based on mineral oils are more efficient than those based on metabolisable oils. However it is linked with stronger local reactions.



**47. Production of antibodies and expression of cytokines mRNA in pig intestinal mucosa during *Trichinella spiralis* infection.** M. Picherot<sup>1</sup>, M. Cote<sup>1</sup>, K. Noeckler<sup>2</sup>, F.J. Serrano<sup>3</sup>, F. Le Guerhier<sup>1</sup>, I. Oswald<sup>4</sup>, P. Boireau<sup>1</sup>, I. Vallée<sup>1</sup>, <sup>1</sup>UMR BIPAR, Maisons-Alfort, France, <sup>2</sup>BfR, Berlin, Germany, <sup>3</sup>Facultad de Veterinaria, Caceres, Spain, <sup>4</sup>INRA, Toulouse, France

In order to study the gut immune response of pigs against *Trichinella* infection, we developed an *ex vivo* model to evaluate the immune response by intestinal mucosa. Pigs were experimentally infected with *T. spiralis* muscular larvae. After 5, 12, 15, 20 or 60 days post infection (dpi), serum, small intestine, jejunum lymph nodes and spleen were collected on animals. Intestinal mucosa was maintained in culture medium for 1 to 3 days. Culture supernatants and serum were analyzed for specific IgG, IgG1 and IgG2 release. Culture supernatants were positive as soon as 15 dpi whereas serum were positive 20 dpi. Only one band of 110 kDa was evidenced in intestinal mucosa and serum, 15 and 20 dpi respectively. On 60 dpi, both of them recognized exactly the same pattern of antigens: 2 single bands (35 and 110 kDa) and 2 double bands (43/46 and 55/59 kDa). IgG1 response was strong in both mucosa and serum whereas IgG2 was highest in serum than in mucosa. mRNA expression of cytokines was analyzed in intestinal mucosa, jejunum lymph nodes and spleen. At 5 dpi, IL10 showed a significant increase only in the mucosa, whereas IFN $\gamma$  was increased in both mucosa and spleen. IL4 and IL6 were not significantly modulated in their expression. We thus evidenced that during *Trichinella* infection of pigs, antibody production firstly appeared in the intestinal mucosa and then at the systemic level. IgG1 response is dominant at intestinal level. IL10 and IFN $\gamma$  production is induced at early stage of infection in pigs. This work was supported by EU contract TRICHIPORSE QLRT-2000-0156.

**48. A strong antibody response against a 49 kDa antigen of *Trichinella spiralis* newborn larva.** M.R. Salinas-Tobón<sup>1\*</sup>, A. Navarrete-Leon<sup>1</sup>, J. Hernández-Sánchez<sup>2</sup>, <sup>1</sup>Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, IPN. Prol. de Carpio y Plan de Ayala S/N, CP. 11340. Mexico, D.F. <sup>2</sup>Departamento de Genética y Biología Molecular, CINVESTAV, IPN. Mexico, D.F.

In *T. spiralis* infection, newborn larva (NBL) stage is the less biologically characterized. Structural findings suggest the presence of cells in the anterior half of NBL which resemble stichocytes of infective larva (ML). The stichosome is an inner structure that may be a potential source of outstanding molecules in the host-parasite interaction as previous studies have shown. In this work we analyzed the antigenic relation of NBL and ML components recognized by rats infected experimentally. Wistar rats were infected with 2000 ML and bled during 61 days at different time intervals (pi). Polyclonal antibodies were obtained in rats immunized with 49 kDa NBL antigen (PoAb). Serum samples and PoAb were tested by ELISA and Western Blot using peroxidase-goat IgG anti-rat immunoglobulin. Soluble *T. spiralis* and excretory-secretory ML antigens (ES) were used. The results showed that Ab to NBL antigens were not detected until day 10, a peak of Ab production was reached by day 14, and Ab levels then decreased from day 19 to 61 pi. Ab response against a 49 kDa NBL component was strong from day 10 to 31, decreased at day 41 and faded by day 61 pi. PoAb did not bind to any soluble ML antigen. In contrast, PoAb bound very weakly to ES components of 62, 49 and 42 kDa and to soluble adult antigens of 62 and 60 kDa. All together these results suggest that the 49 kDa component which is different from TSL-1 antigens, might be a protein transiently expressed during larva maturation in host muscle.

\*Fellow of COFAA

49. **Newborn larva antigen recognition during *Trichinella spiralis* infection.** M.R. Salinas-Tobon<sup>1\*</sup>, M.R. Epitacio<sup>1</sup>, B.E. Mendez-Loredo<sup>1</sup>, D. Esquivel-Aguirre, D.M. Martinez-Abrajan<sup>1</sup>, J. Hernandez-Sanchez<sup>2</sup>.  
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Immunity to newborn larva (NBL) in different host species has shown that serum antibodies (Ab) produced during infection bind to surface antigens (Ag). Ab to surface Ag mediate larval killing via Ab-dependent cell mediated cytotoxicity *in vivo* and *in vitro*. Ab to excretory-secretory and somatic Ag are also produced in the infection but they have not been well characterized and their role in the host interplay has to be determined. Thus, we analyzed the effect of different muscle larvae (ML) doses on Ab production to NBL Ag throughout the infection in rats. Wistar rats were infected with 0, 700, 2000, 4000 and 8000 ML and bled during 31 days at different time intervals (pi). Total immunoglobulin was analyzed by ELISA and Western Blot using soluble NBL or ML Ag. Ab response to NBL showed similar kinetics of different magnitude. A higher Ab response in rats infected with 2000 ML was observed as compared with other doses. Ab were not detected until day 10, a peak was reached by day 14, and then decreased slightly from day 19 to 31 pi. Ab bound at least to three components of 188, 205 and 49 kDa. NBL Ag of 188 and 205 kDa were recognized from day 10 to 26 pi and that of 49 kDa from day 10 to 31 pi. In contrast, Ab level to ML increased from day 12, peaked by day 19 and remained high until the end of the study. An early recognition of 30, 43, 75 and 90 kDa ML Ag was observed whereas the response to those of 45, 52, 61, 65 and 95 kDa (described by others as TSL-1 Ag) occurred late in the infection. These results show the recognition of a restricted set of NBL Ag during *T. spiralis* infection and demonstrate that the highest anti-NBL Ab levels were elicited in rats infected with 2000 ML. \*Fellow of COFAA

50. ***Trichinella spiralis* glycans complexed with monoclonal IgG isotypes interact with mast cell Fc receptors.** S. Thrasher<sup>1</sup>, D. Holowka<sup>2</sup>, and J. Appleton<sup>1</sup>, <sup>1</sup>Baker Institute for Animal Health, Cornell University, Ithaca, NY, 14853; <sup>2</sup>Department of Chemistry and Chemical Biology, Cornell University, Ithaca NY, 14853

Our aim is to define the role of mast cells in expulsion of *Trichinella spiralis* from the intestinal epithelium during a challenge infection. We hypothesize that host immunoglobulins complexed with parasite antigens interact with Fc receptors on mast cells to promote the rapid expulsion of larvae from the intestine. Although correlative evidence suggests that expulsion is dependent on mast cells, the mechanism of expulsion has not been determined. Passive immunization of rats with glycan-specific monoclonal IgG isotypes causes complete expulsion of *T. spiralis* larvae within 24 hours. This protective mechanism only occurs when rats have been previously infected with an unrelated nematode, such as *Heligmosomoides polygyrus*. This infection likely activates some non-specific factor of innate immunity that cooperates with antibodies to cause expulsion. We have determined by flow cytometric analysis that rat IgG1, IgG2a, and IgG2b but not IgG2c immune-complexes bind Fc receptors on RBL-2H3 cells, a rat mast cell line with a mucosal phenotype. Blocking experiments revealed that IgG1 and IgG2a bind to the high affinity IgE receptor (Fc $\gamma$ RI) and a low affinity IgG receptor (Fc $\gamma$ RIIb). IgG2b bound only Fc $\gamma$ RIIb. Additionally, we found that IgG2a immune-complexes trigger RBL-2H3 degranulation and IgG2b does not. Furthermore, even though IgG1 immune-complexes bound Fc $\gamma$ RI, they did not trigger degranulation. Understanding Fc receptor interactions with protective antibodies will help us to determine whether rapid expulsion of *T. spiralis* depends on mast cell activation.

**51. Kinetics of antigen recognition by antibodies produced at the intestinal level in mice infected with *Trichinella spiralis*.** P.B. Garcia-Reyna<sup>1,4</sup>, R. Fonseca-Liñan<sup>1</sup>, L. Yepez-Mulia<sup>2</sup>, R. Salinas-Tobón<sup>3</sup>, I. Vallé<sup>5</sup>, P. Boireau<sup>5</sup>, and M.G. Ortega Pierres<sup>1</sup>. Cinvestav IPN<sup>1</sup>, UIMEIP-Pediatría, IMSS<sup>2</sup>, FES-C, UNAM<sup>4</sup>, Mexico, UMR BIPAR, 94703 Maisons-Alfort, France<sup>5</sup>

Identification of *T. spiralis* antigens (Ag) recognized by antibodies (Ab) at the intestinal level may define molecules useful in the induction of protective immune responses. We have analysed reactivity of Ab produced at the intestinal level to *T. spiralis* Ag. This was done using supernatants (Spn) from cultured explants of duodenum, jejunum and ileum obtained at different times after infection of mice with *T. spiralis*. Antibody reactivity was tested by ELISA, Western Blot (WB) and indirect immunofluorescence (IIF) on parasite sections. ELISA results showed a higher IgA and IgG responses in duodenum and jejunum to L1 and Ad as compared to the ileum. IgM and IgE responses were rather low to all parasite stages. WB results with duodenum Spn showed an early recognition of several L1 Ag (40-210 kDa) by IgA, IgG and IgM while similar components were detected later by IgG and IgM in jejunum and ileum. A set of three Ag (90-210 kDa) in Ad were preferentially recognised by IgA and IgG from duodenum as compared with jejunum. Detection of three NBL Ag (100-150 kDa) was only observed with IgA in duodenum. Almost no reactivity was observed to all Ag when IgE were tested. IIF assays with duodenum Spn showed an early reactivity to surface and internal structures of Ad as compared to the one observed for L1. In contrast jejunum Spn displayed late in the response higher reactivity to L1 structures as compared to Ad ones. Supernatants from ileum showed only a low reactivity to Ad and L1. Almost no reactivity was observed against NBL in this assay. These results showed a differential kinetics in the isotype responses directed against a set of defined Ag of each stage of the parasite providing information for selecting possible candidates for specific induction of protective responses to *T. spiralis*. Supported in part by grants CONACyT (Mexico). G38523-M and ECOS-ANUIES (France-Mexico). M01-A-02N-10.

**52. Are bacillary bands responsible for expulsion of *Trichinella spiralis*?** W. J. Kozek, Department of Microbiology and Medical Zoology, Medical Sciences Campus, University of Puerto Rico, San Juan, PR 00936-5067

The excretory system of the members of the Order Trichurida consists of single glandular cells aggregated to form the bacillary bands. The size, shape and complexity of the bacillary bands varies among the families of this order. Using a combination of light and electron microscopic techniques we have examined the bacillary bands of newborn larvae, muscle larvae, larvae developing in the intestine, and adults of *T. spiralis*. In adults, the bacillary bands begin at the cephalic end of the worms as a row of single cells, each cell being marked by a distinct cuticular pore. The bands gradually increase in width until they accommodate two cells. The pores are patent in the newborn larvae; some cells of the band appear to also have a sensory function. The muscle larvae do not have patent pores, but the pores become patent after the first molt and remain patent in successive larval stages developing in the intestine. The patency of the bacillary bands can be correlated with the life cycle of *T. spiralis*. The bands represent an anatomic weak point in the anatomy of *Trichinella*, which need to be shielded from the enzymatic degradation and immunological responses of the host, and a source of metabolic irritants that can trigger the expulsion mechanisms. This would explain why the intestinal stages of *T. spiralis* inhabit the deeper tissues of the intestine, and not the lumen, and why the newborn larvae need to migrate through the lymphatic and circulatory systems, avoiding exposure to the intestinal enzymes.

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**53. The macrophage mannose receptor involvement in the innate immune response to the infection with parasite *Trichinella spiralis*.** A. Gruden-Movsesijan, Lj. Sofronic Milosavljevic, Institute for the Application of Nuclear Energy-INEP, 11080 Belgrade, Serbia and Montenegro

Macrophage mannose receptor (MR) is a pattern recognition receptor of the innate immune system. Upon binding to the structures bearing mannose, fucose and N-acetylglucosamine on the surface of bacteria, viruses, yeast and parasites, MR trigger mechanisms that are involved in the first line of defense. MR can mediate endocytosis and phagocytosis, as well as activation of macrophages and antigen presentation. Since *T. spiralis* antigens, are rich in oligomannose residues, we investigated if mannose-recognizing receptor, such as MR, participate in the host – parasite interaction. It was shown that MR (either on the surface of macrophages or in the purified form) recognized and bound components of *T. spiralis* muscle larvae. The presence of parasites provoked activation of peritoneal macrophages, which was observed by down-regulation of MR expression, and the stimulation of NO secretion. *In vitro* stimulation of macrophages with *T. spiralis* components resulted in increased NO and IL-6 production. However, while MR was partially involved in stimulation of NO production, MR did not mediate the IL-6 secretion.

**54. IL-10 prevents liver necrosis during murine infection with *Trichinella spiralis*.** S. K. Bliss<sup>1</sup>, A. Alcaraz<sup>2</sup>, and J. A. Appleton,<sup>1,3</sup> James A. Baker Institute for Animal Health<sup>1</sup>, Department of Biomedical Sciences<sup>2</sup>, and the Department of Microbiology and Immunology<sup>3</sup>, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401

Infection with *Trichinella spiralis* rarely leads to significant morbidity. Here we show that IL-10 knockout mice infected with this parasite develop extensive areas of coagulative necrosis in the liver, and newborn larvae are required for lesion formation. Histopathological examination revealed that the hepatic inflammatory infiltrate was mixed but dominated by eosinophils. Accordingly, infected IL-10 knockout mice displayed a marked eosinophilia. IL-10 was expressed during infection in mesenteric lymph node populations and liver tissue. Analysis of cytokine profiles revealed a codominant expression of type 1 and 2 mediators that was enhanced in the absence of IL-10. Additionally, CD11c<sup>+</sup> MHC class II<sup>+</sup> cells were increased in mesenteric lymph nodes of IL-10 knockout mice, suggesting a possible link between IL-10 and dendritic cell trafficking. Nevertheless, there were no significant differences in mortality or parasite burdens between the strains of mice, indicating that IL-10 is necessary to control the host's inflammatory response but does not impact establishment of the parasite. Expression of IL-10 appears to be an adaptation used by the liver to protect itself from damage caused by migrating newborn larvae.

**55. Analysis of the permanence of antibodies against *Trichinella spiralis* in the offspring of mothers infected with the parasite.** C. Maldonado-Tapia<sup>1</sup>, G. Reveles-Hernández<sup>1</sup>, S. Saldívar-Elías<sup>1</sup>, J. Muñoz-Escobedo<sup>2</sup>, A. Moreno-García<sup>1</sup>, <sup>1</sup>Unidad Académica de Biología Experimental, Universidad Autónoma de Zacatecas, Mexico, <sup>2</sup>Unidad Académica de Odontología, Universidad Autónoma de Zacatecas, Mexico

Our interest was to investigate if infection of pregnant rats with different *T. spiralis* dose have any effect in the detection and permanence of specific antibodies against the parasite in their offsprings. For this, groups of 5 pregnant Long Evans rats, 2 and a half months old, were infected with 3,000, 2,000, 1000 or 500 *T. spiralis* muscle larvae. Control groups of non-pregnant, and pregnant non-infected rats were included. The number of new born/ female rat was registered and the humoral immune response against *T. spiralis* was studied by Western blot analysis (WB), in both mothers and their offspring from birth till they were one year old. The number of ML was determined in infected mothers and their offsprings by trichinoscopy and artificial digestion. A group of 10 rats from the offspring, in which antibodies against *T. spiralis* were previously detected, were challenged with the parasite. The data obtained showed that pregnant rats infected with 3000, 2000 or 1000 ML have miscarriages and premature deliveries. In the offspring no parasites were detected although antibodies against *T. spiralis* ML were detected by WB. In rats infected with 500 ML, the number of newborn rats decreased compared to pregnant non-infected rats. In serum samples collected from the offspring, antibodies against *T. spiralis* were detected up to 8 months, however, no protection against *T. spiralis* challenge was observed.

**56. Evaluation of the protection induced by four immunogens against *Trichinella spiralis* infection in experimental trichinellosis.** A. Moreno-García<sup>1</sup>, R. Roman-Díaz<sup>1</sup>, E. García-Mayorga<sup>1</sup>, G. Reveles-Hernández<sup>1</sup>, J. Muñoz-Escobedo<sup>2</sup>, <sup>1</sup>Unidad Académica de Biología Experimental, Universidad Autónoma de Zacatecas, Mexico, <sup>2</sup>Unidad Académica de Odontología, Universidad Autónoma de Zacatecas, Mexico

Several studies have induced protective immune response against *T. spiralis* infection, using different antigenic preparations from the parasite, however, the protection elicited has not been complete and the production of a vaccine has not been achieved. Therefore, our interest was to evaluate the protection induced by four immunogens against *T. spiralis* infection using an experimental model. The immunogens tested were: Total soluble extract (TE) from *T. spiralis* muscle larvae (ML), antibodies against *T. spiralis* ML, *T. spiralis* antigens complexed to antibodies against the parasite and anti-idiotypic antibodies. Groups of mice received four administrations of the four preparations and challenged with *T. spiralis* ML 8 days after the last administration. Serum samples from the immunized animals were obtained and their reactivity analyzed by Western blot. The reduction of ML load was evaluated as an indicator of the protection induced with the immunogens and the integrity of nurse cells was analyzed by Hematoxylin-Eosin staining. The data was statistically analyzed by ANOVA. The results obtained showed that the best protection against *T. spiralis* infection was induced by the immune complexes ( $p > 0.0001$ ), followed by the TE. Nurse cell integrity was affected with these two immunogens. Anti-idiotypes and antibodies anti-*T. spiralis* also induced protection ( $p > 0.001$ ), however, no effect was observed on nurse cells.

**57. Viability of *Trichinella* larva in outside of host's body in different environmental conditions.**

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Sylvatic trichinellosis is prevalent in different parts of Iran in wild life. There is two strains of *trichinella* in the country according the susceptibility of cotton rat, one in the northern part identified as *T.spiralis*, another in the southern part (tropical region) as *T. nelsoni*. In this study *Trichinella* larvae obtained by digesting of experimentally infected white mice, were kept in 4<sup>0</sup>C refrigerator and in different intervals a number of these larvae were fed to white mice in order to see viability of larvae. The experiment revealed that up to 20 days the larvae keep their viability and infectivity. In another experiment the undigested muscles containing larvae, were kept in temperatures 14-25<sup>0</sup>C in the laboratory condition. These larvae were alive and their viability and infectivity were protected up to 7days. For all experiments the control groups were used. The significance of this finding is discussed due to prey and predator relation.

**58. Epidemiology of *Trichinella* in wildlife in the Netherlands and the first isolation of *T. pseudospiralis*.**

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Epidemiological studies have been carried out in wildlife in several non-endemic countries to eventually estimate the risk of a *Trichinella* infection for domestic animals and humans. In the Netherlands, surveys in foxes revealed higher prevalence compared to 20 years ago and the presence of *T. britovi*. In addition, a serological monitoring program in wild boars has been carried out for several years now to determine prevalence trends in time. Wild boar populations are located in the central and in the southern part of the country. The serological prevalence of *Trichinella* infections using an ES antigen based ELISA ranged from 4% to 6 % using a cut-off based method. Risk factor analysis showed that the prevalence was highest in the central part of the Netherlands and in addition, age was associated with infection. No significant differences in sero-prevalence were seen over the years. To evaluate the results of serological testing in wild boars, between 2003-2004 a study was carried out in 107 wild boars to estimate the risk for public health of a serologically positive animal by simultaneously testing 45 grams of diaphragm of these wild boars. Sero-prevalence in these animals tested was 7.4%. Only one animal was positive in the digestion method with 1,32 LPG. Molecular identification of the larvae using the 5S tandemly repeated intergenic spacer based PCR showed an 800 bp fragment. DNA sequencing analysis of the PCR product showed that the 3-prime end was homologous with the DNA sequence of the non-encapsulated *T. pseudospiralis*, 522 bp PCR product. Hence, this was the first isolation of *T. pseudospiralis* in a wild boar in the Netherlands. The infection load of the animal revealed a potential human infection risk.

**59. The influence of a high prevalence of sylvatic trichinellosis on the domestic dog population in Finland.** Leena Oivanen<sup>1</sup>, Anu Näreaho<sup>1</sup>, Saija Jokela<sup>1</sup>, Ulla Rikula<sup>2</sup>, Ray Gamble<sup>3</sup>, Antti Sukura<sup>1</sup>, <sup>1</sup>Department of Basic Veterinary Sciences, Faculty of Veterinary Medicine, University of Helsinki, <sup>2</sup>National Veterinary and Food Research Institute, Helsinki, Finland<sup>3</sup>

The influence of a high endemic *Trichinella* infection of sylvatic hosts in Finland was studied in domestic dogs. A total of 727 dog serum samples were analyzed by ELISA with ES-antigen. Additionally, muscle samples from the front leg extensor muscles of 102 dogs were tested for the presence of parasites by HCl-pepsin-digestion. Canine serum samples originated from the University Veterinary Hospital, (244 sera) and from a serum bank of the National Veterinary and Food Research Institute (465 sera); the latter group represented healthy dogs mostly from southern Finland. As controls, serum samples were collected from 18 experimental dogs. Muscle samples were obtained from autopsy material sent to the Section of Veterinary Pathology. *Trichinella* spp. was isolated from 1 dog (1%) with a very low infection level. In both groups of serum samples, high OD% was found by ELISA. By K-mean cluster analyses 4.9% of the hospitalized and 8.6% of healthy ones dogs were classified positive. The sex of the tested dogs was not correlated with *Trichinella* seropositivity nor was the breed of dog; however, older age was associated with higher OD values in the serum bank group of dogs. These results show that dogs are exposed to *Trichinella* spp. in Finland. The fact that hunting breeds did not show higher OD% than other breeds may be explained by the Finnish life style in which families spending their leisure time at the shore and at cottages with their dogs, regardless their breed.

**60. *Trichinella britovi* in sylvatic carnivores of Guinea Conakry (West Africa).** E. Pozio<sup>1</sup>, P. Pagani<sup>2</sup>, G. Marucci<sup>1</sup>, L. Rossi<sup>3</sup>, G. La Rosa<sup>1</sup>, <sup>1</sup>Istituto Superiore di Sanità, Rome, Italy; <sup>2</sup>Veterinaires Sans Frontieres, France; <sup>3</sup>Department of Animal Productions, Epidemiology and Ecology, University of Turin, Italy

In West Africa, *Trichinella* infection was detected in wildlife in the sixties. A *Trichinella* isolate from one of these animals showed a low infectivity for swine and rodents; however at that time, the parasite strain was lost preventing its identification at the species and/or genotype level by biochemical and/or molecular studies. We have investigated on the presence of *Trichinella* infection in wildlife of West Africa and on the identification of the etiological agent. The study area was the North-West region of Guinea Conakry. Samples were obtained from the anterior tibial muscle of animals either collected by local hunters (94%) or road-killed (6%) between November 2001 and June 2003. Samples were preserved fresh at +4°C or in 1% merthiolate solution. Of 160 examined animals, 158 were mammals belonging to the family Suidae (12), Canidae (5), Viverridae (126), Felidae (5), Galagonidae (2), Cercopithecidae (7) and Hominidae (1); a hooded vulture (*Neophron monachus*) and a monitor lizard (*Varanus niloticus*) were also examined. *Trichinella* larvae were detected in 4 animals belonging to 3 species of the family Viverridae, namely the Pardine genet (*Genetta pardina*), the African civet (*Civettictis civetta*) and the African palm civet (*Nandinia binotata*). All larvae have been identified as *T. britovi*. The findings of *T. britovi* in sylvatic carnivores in West Africa at a latitude of about 12°N force us to reconsider the distribution of encapsulated species in the African continent and to take into account the phylogenetic relationship with *Trichinella* T8, a *T. britovi*-related genotype detected in wildlife of Namibia and South Africa. This work was funded by the project of the Istituto Superiore di Sanità, entitled "Epidemiologia molecolare e diagnostica delle infezioni parassitarie a carattere zoonotico" contract C3MO.

**61. Outbreak of trichinellosis associated with consumption of Walrus in West Greenland.** L.N. Møller<sup>1,2</sup>, E. Petersen<sup>1</sup>, C.M.O. Kapel<sup>2</sup>, M. Melbye<sup>3</sup> and A. Koch<sup>3</sup>, <sup>1</sup>Department of Bacteriology, Mycology & Parasitology, Statens Serum Institut, Copenhagen, <sup>2</sup>Danish Centre for Experimental Parasitology, Frederiksberg, <sup>3</sup>Department of Epidemiology Research, Statens Serum Institut, Copenhagen

Trichinellosis is a problem in humans in the Arctic as well as in other climates. The Inuit population of the Arctic has always been at risk of acquiring infection with *Trichinella*, and severe outbreaks have been recorded in Alaska and Canada. In West Greenland, a number of large outbreaks took place during the 1940'ies and 1950'ies when in total 420 cases of trichinellosis were registered of which 37 people died. Since then only sporadic cases have been registered. Here we describe an outbreak of infection with *Trichinella* spp. after consumption of presumably infected walrus meat from the west coast of Greenland. Six persons had eaten of the meat, two males and four females, age range 6-47. Using ELISA and Western blot analysis of *Trichinella*-specific IgG antibodies against excreted/secreted antigen and synthetic tyvelose antigen, respectively, four of these persons were found sero-positive for *Trichinella* antibodies, three of them having clinical symptoms of trichinellosis. By retesting 12-14 months later one of the two sero-negative persons had sero-converted, probably due to a new, unrelated infection. Consumption of game meat (walrus) in Greenland still poses a risk of acquiring trichinellosis, but can be prevented by public health measurements.

**62. Human trichinellosis in Greenland.** L.N. Møller<sup>1,2</sup>, S. Andersen<sup>3</sup>, M. Melbye<sup>4</sup>, E. Petersen<sup>1</sup>, C.M.O. Kapel<sup>2</sup>, P. Laurberg<sup>3</sup> and A. Koch<sup>4</sup>, <sup>1</sup>Department of Bacteriology, Mycology & Parasitology, Statens Serum Institut, Copenhagen, <sup>2</sup>Danish Centre for Experimental Parasitology, Frederiksberg, <sup>3</sup>Department of Medical Endocrinology, Aalborg Hospital Nord, Aalborg. <sup>4</sup>Department of Epidemiology Research Statens Serum Institut, Copenhagen, Denmark

Trichinellosis is a well-known problem in Greenland. In West Greenland a number of large outbreaks took place during the 1940'ies and 1950'ies, but since then only sporadic cases have been registered. It is unknown whether the decrease in trichinellosis cases reflects the general transition in Greenland towards a more western lifestyle with less consumption of meat from wildlife, or whether it reflects insufficient case registration. The aim of the present study was to determine the prevalence of trichinellosis in Nuuk, the capital of Greenland, where the lifestyle is much western, and in the main town Tasiilaq and settlements in Ammassalik district on the east coast of Greenland, where more traditional food is still eaten, in particular in the settlements. Blood samples from in total 86 persons collected in 1981 and from 533 persons collected in 1998 in Nuuk and Ammassalik district were tested for *Trichinella*-specific IgG antibodies using ELISA and Western blot analyses. We found for 1981 a trichinellosis prevalence of 8.7% in Nuuk and 23.8% in Ammassalik district, and for 1998 a prevalence of 5.2% in Nuuk and 19.8% in Ammassalik district. Persons living in the settlements of Ammassalik district had higher antibody prevalence than persons living in the town of Tasiilaq. As life style in the settlements is more traditional compared both with Tasiilaq and Nuuk, these results indicate that the decline in trichinellosis cases in the 19<sup>th</sup> century most likely reflects the transition from a traditional lifestyle to more western dietary habits.



63. **Molecular epidemiology of *Trichinella* spp. in three Baltic countries: Lithuania, Latvia and Estonia.** A. Malakauskas<sup>1</sup>, V. Paulauskas<sup>2</sup>, P. Keidans<sup>3</sup>, T. Järvis<sup>4</sup>, C. Eddi<sup>5</sup> and C.M.O. Kapel<sup>6</sup>, Lithuanian Veterinary Academy<sup>1</sup>, Lithuania, Kaunas 47181, Lithuanian National Veterinary Laboratory<sup>2</sup>, Lithuania, Vilnius 2021, Latvia University of Agriculture<sup>3</sup>, Latvia, Jelgava 3004, Estonian Agricultural University, Estonia<sup>4</sup>, Tartu 51014, Animal Health Service<sup>5</sup>, Food and Agriculture Organization of the United Nations, Italy, Rome C-528, Danish Centre for Experimental Parasitology<sup>6</sup>, Royal Veterinary and Agricultural University, Denmark, Frederiksberg C 1870

Meat of domestic pigs and wild boars has been the significant source of emerged human trichinellosis in the three Baltic States over the past two decades. However, occurrence of the parasite in main wildlife reservoirs and transmission of *Trichinella* spp. in domestic and sylvatic cycles has not been properly investigated. An epidemiological survey carried out from 2000 to 2002, demonstrated considerably higher endemicity of *Trichinella* in main sylvatic reservoirs (28.9 - 42% in foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*)) in all three countries than previously reported. Raccoon dogs, which are introduced species in the Baltics, harbored significantly higher larval burdens than foxes, indicating its important role in the transmission of trichinellosis today. Identification of *Trichinella* larvae from more than 500 sylvatic and domestic animals revealed 4 *Trichinella* species sympatric in relatively small area and several as the first records for the respective countries. Sylvatic *T. britovi* was found in domestic pigs in Lithuania and Latvia (16% and 57.1%, respectively) and only in these countries domestic *T. spiralis* was detected in sylvatic animals in areas where domestic trichinellosis was registered. The study suggests that transmission of *Trichinella* between domestic and sylvatic cycles in Lithuania and Latvia is favored by improper human behavior, e.g. pig and slaughter waste management.

64. ***Trichinella nativa* in a black bear from Plymouth, New Hampshire.** D.E. Hill<sup>1a</sup>, H.R. Gamble<sup>2</sup>, D.S. Zarlenga<sup>1b</sup>, Cathleen Coss<sup>1a</sup>, and J. Finnigan<sup>3</sup>; <sup>1</sup>USDA, ARS, ANRI, <sup>a</sup>Animal Parasitic Diseases and <sup>b</sup>Bovine Functions and Genomics Laboratory, Beltsville, Maryland; <sup>2</sup>National Academy of Sciences, Washington, D.C., and <sup>3</sup>The Food Safety Microbiology Unit, New Hampshire Public Health Laboratories, Concord, New Hampshire

A case of trichinellosis was identified by the Public Health Laboratories in Concord, New Hampshire. The suspected etiologic agent was muscle larvae in undercooked meat from a black bear killed in Plymouth, New Hampshire in October, 2003. Meat from the bear was frozen at -20°C, and 1.5 kg was sent to the Animal Parasitic Diseases Laboratory in December, 2003 and stored at -20°C for later analysis. In January, 2004, a 600g sample of the meat was thawed, digested in pepsin (1%):HCl (1%), and intact, coiled, and motile L1 were recovered (366 larvae per gram of tissue) and used to infect mice and pigs. Pig tissue (100g) was collected from 15 muscle groups 5 weeks post infection, from animals inoculated with 2500 or 10,000 L1. One larva was recovered from throat muscle of 1 pig (1 year in age, n=4) infected with 10,000 larvae. Numerous viable larvae were recovered from all 4 pigs that were 3 months old at the time of infection (n=4). At approximately the same time, viable L1 were also isolated from frozen meat from 2 additional black bears, 1 harvested in Clinton County, New York and obtained through a custom slaughterhouse, and the other from Timmons, Ontario Canada, that was field dressed and transported to Tennessee. Multiplex PCR analysis of each of the 3 *Trichinella* isolates revealed a single 127 bp amplicon, indicative of *Trichinella nativa*. These are the first reports of freeze resistant *T. nativa* within or proximal to continental United States borders.

65. **The evolution of Trichinosis in men studied on necroptical data (Summary).** Debora Cristea<sup>1</sup>, Eugenia Cristea<sup>2</sup>, Gh. Cristea, The Medicine and Pharmacy University Carol Davila, Bucharest, the VI-th year student, <sup>2</sup> The Individual Medical Cabinet, general doctor, N. Titulescu street, bl. T3, 336200-Vulcan, Romania, <sup>3</sup>The Veterinary District Vulcan, N.Titulescu street, No 42, 336200- Vulcan, Romania

Trichinosis in men evolves without symptoms or with uncertain clinical signs and death is attributed to other causes. Between 1993-2003, we examined 169 bodies (130 men and 39 women) through autopsy – these people died from causes other than Trichinosis. We found *Trichinella* larvae in 40 people (30 men and 10 women), with ages between 2-74 years. Causes of death included: 8 deaths from accidents, 1 from aggression, 13 by asphyxia, 7 from heart attack and 11 from internal disease. Examining 12 body regions from 3 human bodies, we estimated the intensity of infection in tongue (1156.3 larvae/gram), diaphragm (1048.6 larvae/gram), the masseter (896 larvae/gram). Diminished intensity was found in the haunch muscles and in the external pelvis muscles (1.3 larvae/gram). In the superior region of the body larval numbers are greater than in the inferior areas. We examined the muscles near the tendon insertion. Numbers of larvae decreased by 60% distally from the insertion of the tendon.

66. **The occurrence and distribution of *Trichinella* spp. in Canadian wildlife.** Alvin Gajadhar, Lorry Forbes, and Terry Steeves-Gurnsey, Centre for Animal Parasitology, Saskatoon Laboratory, Canadian Food Inspection Agency, Saskatoon, Sask., S7N 2R3, Canada

*Trichinella*, originally described as a monotypic genus, is now recognised to occur in North America as five distinct species each with a somewhat defined host and geographic distribution. *Trichinella spiralis*, once common in domestic pigs, is rarely seen on this continent. *Trichinella nativa* occurs in northern regions while distributions of *Trichinella murrelli* and T6 are apparently sporadic, and *T. pseudospiralis* has been rarely reported. This report describes results from an ongoing long-term survey being conducted to determine the identity and distribution of *Trichinella* spp. in Canadian wildlife. Muscle samples from the carcasses of various carnivorous or omnivorous mammals and birds from the eastern, western and northern regions of Canada were tested using the pepsin/HCl digestion method. *Trichinella* larvae recovered were identified to species using a standard multiplex PCR assay which amplifies regions of the large subunit ribosomal DNA and internal transcribed spacers 1 and 2. *T. nativa* was found to occur commonly in a variety of hosts in the north, and occasionally cougars and bears were found to harbour *T. murrelli* and T 6. Intra- and inter-specific genetic differences among *T. nativa*, *T. murrelli* and T6 from different regions and from different hosts are being examined. The results of this research will help to clarify the identity and distribution of *Trichinella* spp. and isolates in North America.

67. **Trichinellosis of wild mammals in northwest Ukraine.** I. A. Akimov, J. M. Didyk I. I. Schmalhausen Institute of Zoology NAS of Ukraine, B. Khmel'nits'kogo, 15, Kyiv-30, MSP, Ukraine, 01601

A little is known about current prevalence of the trichinellosis of wild animals in Ukraine, the data received in 1950-1970 years are sometimes contradictory. Moreover, special investigations of ungulates in Ukraine were not carried out. Therefore our work was aimed both to studying the prevalence of the trichinellosis of wild ungulates and carnivores (wild boars, roedeers, deers, wolves, foxes, otters and martens) in Ukraine and collecting *Trichinella* isolates for molecular studies. The material was collected in Chernigiv, Kyiv, Zhitomir, Rivno, Ternopil and Carpathian regions during the two hunting seasons: 2002/2003 and 2003/2004 years. The muscle tissues were tested for *Trichinella* larvae by trichinoscopy and artificial digestion methods. The obtained larvae were preserved in 75 % ethanol for the future research. Muscle samples of a total 114 wild mammals were examined: 43 wild boars, 21 roedeers, 5 deers, 10 wolves, 23 foxes, 5 otters and 7 martens. The *Trichinella* infection was found in two wild boars, two foxes and two wolves. The intensity of *Trichinella* invasion of wolves and foxes was rather high: 5 – 8 larvae per 1 gram of muscle tissue. The wild boars were infected weakly, though there was registered one case of the people infection due to eating of the meat of infected animal. Only one bear was tested and it also was infected with *Trichinella* sp. No *Trichinella* invasion was found in all studied roedeer, otters and martens.

68. **Trichinellosis in wild and domestic animals in Poland.** W. Cabaj, B. Moskwa, K. Pastusiak, J. Bien, A. Malczewski, W. Stefanski Institute of Parasitology of the PAS, 00-818 Warsaw, Twarda 51/55, Poland

Four species: *Trichinella spiralis*, *T. britovi*, *T. nativa*, *T. pseudospiralis* have been identified in Europe. In Poland, *T. spiralis* and *T. britovi* have been detected in red foxes by Nowosad and Pozio (1998). The aim of the investigation has been to study distribution of *Trichinella* species in wildlife and domestic pigs from Poland. Muscles were collected from red foxes (*Vulpes vulpes*), wolves (*Canis lupus*) and wild boars (*Sus scrofa*) killed by hunters in different regions of Poland from 1995 to 2003 and from slaughtered domestic pigs in which larvae of *Trichinella* (ML) were detected. ML were collected after standard artificial digestion. The molecular identification of *Trichinella* larvae at the species level has been carried out at the ITRC in Rome, Italy. Out of 72 *Trichinella* isolates from red foxes; 47 resulted *T. britovi*, 6 *T. spiralis*, 4 mixed infection with both species and 15 were not identified. On 6 examined wolves from Bieszczady region 3 animals resulted positive for *T. britovi* larvae. Out of 88 isolates from wild boars; 20 resulted *T. britov*, 64 *T. spiralis*, 2 mixed infection and 4 were not identified. Out of 21 isolates from domestic pigs; 20 resulted *T. spiralis* and 1 *T. britovi*. The study shows that two *Trichinella* species are present in Poland; *T. britovi* as the important etiological agent of sylvatic trichinellosis especially in carnivores in almost the whole territory of Poland. *T. britovi* is also present in wild boars but in minority and occasionally in domestic pigs. This work was supported by research grant No. 6 P04C 02218 from the State Committee for Scientific Research, Warszawa, Poland and by the EU project "TRICHIPORSE" (contract QLK1-CT-2001-01156).

69. **Natural and synanthropic *Trichinella* infection in the Central Region of Russia.** O.N. Andreyanov, A.S. Bessonov, K.I. Skryabin Institute of Helminthology, Moscow, Russia.

The Central Region of Russia in particular the Moscow and Ryazan Areas with population of more 16 millions of humans have been studied insufficiently on prevalence of *Trichinella spiralis* in wild animals as a very important source of *Trichinella* causative agent for domestic animals and humans. The aim of this work is to fill this existing gap. Carcasses of animals for investigations were obtained from hunters, fur-breeders and deratisators. One examined the most affected by *T. spiralis* and easy of access muscles (masticatory in rodents and insectivores, constrictors of hind legs in carnivores, crus of diaphragm in swine, wild boars and humans) using compressorium trichinelloscopy (Reissmann, 1908) or artificial digestion (Vladimirova, 1965). One examined 2286 muscle samples originated from 28 species of animals and humans including 738 samples from humans, 426 samples from carnivores attributed to 10 species, 874 samples from rodents of 16 species and 248 samples from cloven-footed animals of 2 species. *T. spiralis* larvae were found in 2 humans (0,27%), in 1 of 41 examined light polecats (*Mustela eversmanni*) (2,44%), in 8 of 34 common martens (*Martes martes*) (23,53%), in 5 of 27 foxes (*Vulpes vulpes*) (18,52%), in 1 of 16 domestic cats (*Felis catus*) (6,25%), in 1 of 8 racoon-like dogs (*Nyctereutes procyonoides*) (12,5%), in 2 of 108 grey rats (*Rattus norvegicus*) (1,85%), in 1 of 67 water voles (*Arvicola terrestris*) and in 1 of 7 Russian desmans (*Desmana moschata*) (14,29%). One noted reduction of the rate of *T. spiralis* infection in inhabitants of Moscow from 3,4% in 60-years of the last century (Bessonov, Kaporceva, 1967) to 0,27% in present or by 12,6 times. Among animals infected by *T. spiralis* the representatives of natural biocenosis predominate compared with those ones of synanthropic biocenosis including a human (5 species compared with 4 ones). For the first time the infection of Russian desman (*Desmana moschata*) was revealed.

70. **The prevalence of *Trichinella britovi* among different populations of wolves in Croatia.** R. Beck<sup>1</sup>, J. Kusak<sup>2</sup>, A. Marinculic<sup>1</sup>, D. Huber<sup>2</sup>, A. Beck<sup>3</sup>, E. Pozio<sup>4</sup>G. Marucci<sup>4</sup>, Department of Parasitology<sup>1</sup>, Department of Biology<sup>2</sup>, Department for Pathology<sup>3</sup>, Veterinary Faculty, University of Zagreb, Heinzelova 55, 10000, Zagreb, Republic of Croatia; Laboratory of Parasitology<sup>4</sup>, Istituto Superiore di Sanità, viale Regina Elena 299, 00161, Rome, Italy

The aim of the present study was to investigate the prevalence of *Trichinella* infection in wolves (*Canis lupus*) among geographically distinct populations permanently present on 17468 km<sup>2</sup> with 120 to 170 individuals. The studied regions were Gorski Kotar, Lika and Dalmatia. Muscle samples were collected from 33 wolves killed between 1996 and 2004 and analysed by artificial digestion. The muscle larvae were also typed by the multiplex PCR method. *Trichinella britovi* was the only species with the prevalence of 27,27 %. Infection rate was from 0,3 to 45,9 larvae per gram. Geographical distribution of infected animals varies. No infected animals were found in the region of Gorski Kotar. The wolves from the region of Lika were all found infected. The prevalence of *Trichinella* in wolves from Dalmatia was 13,04%. The wolf that was killed near Zagreb was also found positive. In this article we have compared eating habits, density of wolves, and possible impact of people on *Trichinella* distribution among different populations of wolves in Croatia.

**71. Anthelmintic activity of a novel 2-(trifluoromethyl) benzimidazole derivative in experimental trichinellosis.** J. González<sup>1</sup>, O. Soria<sup>2</sup>, I. V. Cruz<sup>3</sup>, A. Hernández-Campos<sup>3</sup>, F. Hernández-Luis<sup>3</sup>, R. Castillo<sup>3</sup> and L. Yépez-Mulia<sup>1</sup>. UIMEIP-IMSS, Mexico,<sup>1</sup> Sistemas Biológicos, UAM-Xochimilco, México<sup>2</sup>, Facultad de Química, UNAM, México<sup>3</sup>

The Benzimidazole-2-carbamates, such as albendazole (**ABZ**) and mebendazole, have been used to treat *Trichinella* infections. However, because of their insolubility, high doses and long treatments are required to reach optimal plasma levels. In an attempt to have other anthelmintic molecules with better solubility and absorption characteristics, we have synthesized a novel 2-(trifluoromethyl) benzimidazole derivative. We determined the anthelmintic activity of this new benzimidazole and **ABZ** against the adult and the muscle phase of *T. spiralis*. For this, BALB/c mice were infected per os with *T. spiralis* muscle larvae in a dose of 500 larvae/mouse and orally treated with the drugs at 75 mg/kg b.w. ( $\mu\text{M}$  equivalent to **ABZ**). For enteral studies, the drugs were administered at day 3 pi and animals were killed at day 6 pi; for parenteral studies, the drugs were given at day 28 pi for 7 consecutive days and animals were killed 7 days after the last administration of the drugs. Control groups included infected animals that were dosed with the same solution used to suspend the drugs. The percentage of reduction of worm load was referred to the non-treated group. The novel 2-(trifluoromethyl)benzimidazole derivative had similar efficacy than **ABZ** against the adult worm, reducing 80% of the parasite load. However, against the muscle larvae stage, a decrease in its efficacy was observed compared to **ABZ** (40 % and 70% respectively). Further studies about the anthelmintic activity of this novel compound against *T. spiralis* are in progress.

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**72. Human trichinellosis: presence of specific IgE and IgG4 in sera from patients undergoing the acute and chronic phases of the infection.** M.A. Calcagno, M.A. Forastiero, M.L. Verzoletti, S.N. Costantino and S.M. Venturiello, Chair of Immunology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina, 1113

Taking into account the role of IgE and IgG4 in helminth infections, the aim of this work was to analyze the presence of these isotypes and its modulation in the antigenic recognition of the excretory-secretory products of the muscle larva (ML-ESP) in acute and chronic human trichinellosis. Forty-nine sera from patients belonging to trichinellosis outbreaks arisen in Argentina were analyzed by indirect ELISA and western blot (WB). These sera were obtained at 2 months (acute phase, AP; n=17) and at 1-3 years post-infection (chronic phase, CP; n=32). Anti-human IgE and anti-human IgG<sub>4</sub> conjugated to peroxidase or biotin were employed. Results showed that: 1) Specific IgE and IgG<sub>4</sub> were detected in 100% of the sera; 2) By WB, the IgE recognized the bands of  $\cong$ 116, 97, 66, 55 and 45 kDa being variable the recognition of the  $\cong$ 36 and 29 kDa. Patients belonging to the CP did not react against the  $\cong$ 29 kDa band. The IgG<sub>4</sub> reacted against the bands of  $\cong$ 45 and 55 kDa being variable the recognition of the  $\cong$ 66 and 97 kDa bands. The bands of MW  $>$   $\cong$ 55 kDa were recognized by a greater percentage of sera from the CP; 3) Levels of specific IgE and IgG<sub>4</sub> did not change over time, even when the levels of total specific antibodies detected by ELISA decreased from the AP to the CP. This work demonstrates the simultaneous presence of IgE and IgG<sub>4</sub> in both the AP and CP. Both isotypes recognize the components of  $\cong$ 45 and 55 kDa, specific for the immunodiagnosis of *T. spiralis* infection. A modulation in the antigenic recognition of the ML-ESP was observed.

**73. Human trichinellosis during pregnancy: a case report.** S.M. Venturiello, G.G. Nuñez, M.I. Calvo\*, S.N. Costantino, M. Derdoy\* and T. Gentile, Chair of Immunology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires (1113), \*Pedro Solanet Municipal Hospital, (7150) Ayacucho, Buenos Aires, Argentina

In this work we describe the clinical, parasitological and serological features of a 22 years old pregnant woman belonging to a human trichinellosis outbreak occurred in Argentina. The patient was in the 3<sup>rd</sup> trimester of pregnancy. The infection was acquired through the ingestion of pork products of a commercial source which parasite burden ranged from 0.066 to 0.85 muscle larvae (ML) per gram. By day 11 post-infection she presented myalgia and eosinophilia of 17%. At this time three serological methods were performed: IFA (using cryostat sections of free ML) which rendered a negative result and ELISA and WB (employing ML-ESP) which were positive. Antihelminthic treatment was not administered. The woman gave birth a healthy baby at the 40<sup>th</sup> week of gestation by cesarean surgery. By this time the IFA, ELISA and WB showed high antibody titres in both serum and placental extraction serum. No specific antibodies were detected in umbilical cord blood. Both the umbilical cord and one-third of the placental tissue were subjected to peptic digestion to find no larvae. Three months after birth the baby was serologically tested, being positive for all tests and also displaying in WB the typical specific bands of *T. spiralis* ESP: 45, 55 and the band of 66 kDa. Nevertheless, final confirmation of congenital infection will be carried out after six months after birth, time at which complete clearance of maternal IgG will have taken place. Moreover, and similarly to our observations in the rat model, the mild course of the disease in this pregnant woman might account for a synergism between the Th2-skewed immune responses found in trichinellosis and in pregnancy.

**74. Treatment with albendazole (Eskazole) in trichinellosis.** R. Olariu<sup>1</sup>, L. Negrutiu<sup>1</sup>, I. Iacobiciu<sup>1</sup>, G. Darabus<sup>2</sup>, A. Koreck<sup>3</sup>, and I. Marincu<sup>1</sup>, University of Medicine and Pharmacy Timisoara, Romania<sup>1</sup>, Faculty of Veterinary Medicine Timisoara, Romania<sup>2</sup>, Faculty of Medicine, University of Szeged, Hungary<sup>3</sup>

Human infection with *Trichinella spiralis* is a zoonosis, in which the parasitic infection is initiated upon consuming uncooked contaminated meat. The nematode may cause severe forms of the parasitic disease, considered an emergency in the medical practice. We have studied the efficacy and tolerance of Albendazole in the treatment of trichinellosis. We investigated 28 adults hospitalized in the clinic of infectious diseases from Timisoara. We have identified 8 asymptomatic cases, 14 patients with moderate forms of disease, and 6 severe forms of trichinellosis. Diagnosis was made using epidemiological criteria, the clinical symptoms and laboratory tests. 28 patients were treated with Albendazole (Eskazole) 400 mg, twice daily, for seven days. After 3 days of therapy the fever decreased in 25 patients (89.28%), and the values of eosinophils were significantly reduced in 23 patients (82.14%) after 7 days of treatment. No side effects were noted. In conclusion Albendazole proved to be a successful alternative in the therapy of trichinellosis.

**75. Cell-mediated immune response to *Trichinella* in persons with a old history of trichinellosis.** M.A. Gomez Morales, A. Ludovisi, E. Pozio, Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy

In humans, *Trichinella* larvae can retain their infectivity in striated muscles for up to 30 years. Consequently, the resulting persistent antigenic stimulation may lead to the polarisation of T-cell subset populations and to the modification of the immunoregulatory states. *Trichinella spiralis* crude worm extract (CWE) induces proliferation in human peripheral blood mononuclear cell (PBMC) with an increase in CD8<sup>+</sup>CD3<sup>+</sup> lymphocytes and a type 2 cytokine pattern during the muscular invasion. The objective of the present study was to characterise the phenotype and the functionality of the long term cell-mediated immunity in persons with an old history of trichinellosis. PBMC were collected from 7 persons, who have been infected 7 (2), 15 (1) and 42 (3) years, previously, and from 1 person who suffered a re-infection 7 years after the primary infection. The results show that CWE is able to induce proliferation of PBMC collected up to 42 years post infection (p.i.). After antigen stimulation, activated cells were constituted by a percentage of lymphocytes, which varies from 40%, 7 years p.i., to 20% and 15%, 15 and 42 years p.i., respectively. Seven years p.i., the subpopulation was mainly constituted by CD8<sup>+</sup>CD3<sup>+</sup> T-cells producing mostly IL-4. Fifteen and 42 years p.i., the subpopulation was mainly constituted by CD3<sup>+</sup>CD4<sup>+</sup> T-cells producing mostly IFN- $\gamma$ . In the reinfected person, the subpopulation (mainly CD3<sup>+</sup>CD4<sup>+</sup>RO T-cells producing IFN- $\gamma$ ) was higher (73%) than that observed after the primary infection (60%). These results indicate that *Trichinella* is able to induce a long lasting memory T-cell response for more than 4 decades p.i.. This work was funded by the project "Studio della risposta immune ad agenti zoonotici: *Cryptosporidium* e *Trichinella*", code 157 of the Istituto Superiore di Sanità.

**76. Improvement of the albendazole efficacy against encapsulated larvae of *Trichinella spiralis* in a murine model using a Hydroxypropyl- $\beta$ -Cyclodextrin liquid formulation.** M.A. Gomez Morales, A. Casulli, E. Pozio, Istituto Superiore di Sanità, Rome, Italy

The aim of the present work was to improve the antihelminthic efficacy against *Trichinella* larvae present in the muscles by an increase in the drug absorption, using a new Albendazole (ABZ) formulation with Hydroxypropyl- $\beta$ -Cyclodextrin ( $\beta$ -CDS). Sixty Balb/c female mice, were infected per os with 120 *Trichinella spiralis* larvae (L1). The antihelminthic efficacy was evaluated comparing 3 groups of infected mice treated with ABZ, ABZ+ $\beta$ -CDS, and not treated mice (controls). Forty days after infection, mice were treated daily per os for 2 weeks as follows: 1) 20 mice with 15mg/kg/day of ABZ in water suspension; 2) 20 mice with 15mg/kg/day of ABZ in a 200 mM solution of  $\beta$ -CDS with citric acid; and 3) 20 mice with saline. The drug efficacy on encysted larvae was evaluated through 2 parameters: i. the number of larvae detected in muscles of the 3 mouse groups collected a week after treatment; and ii. the infectivity of these larvae for other mice. Recovered larvae were used to infect 3 new groups of mice. After treatment, the number of muscle larvae recovered from mice, which had received ABZ or ABZ+ $\beta$ -CDS, showed a mean reduction of 1.8% (39,888 $\pm$ 368) and 19.5% (32,699 $\pm$ 242), respectively, in comparison to the number of larvae recovered from control mice (40,620 $\pm$ 377.2). Larvae collected from treated animals showed a mean reduction of their infectivity of 71.8% (400 $\pm$ 37) and 96.2% (54 $\pm$ 7) for those from ABZ-treated mice and ABZ+ $\beta$ -CDS-treated mice, respectively, in comparison to the infectivity of larvae recovered from control mice (1,418 $\pm$ 160). The statistical analysis (one-way ANOVA test) showed a significant difference between the number of larvae collected from ABZ+ $\beta$ -CDS-treated mice vs control mice and ABZ+ $\beta$ -CDS-treated mice vs ABZ-treated mice (P<0.001 in both cases).

**77. The concept of primary and secondary trichinellosis.** G. Enache<sup>1</sup>, D. Panaitescu<sup>2</sup>, V. Babes Hospital Bucharest<sup>1</sup>; Cantacuzino Institute Bucharest<sup>2</sup>

On the basis of a study made for a period of 8 years (1992-2000) in "Dr.V.Babes" Clinical Hospital of Infectious and Tropical Diseases, we consider it accurate to include within the pathology of trichinellosis both what we have called secondary trichinellosis, observed in the ill person for long periods of time (by hospitalization). The data gathered during this research certainly prove that, generally in our country and all over the world, trichinellosis is monitored only in the phase that we call primary, but the suffering of the ill person that surpasses the primary phase extends over several years. Chronic illness presents various symptoms and laboratory data (serology, LDH, CPK, hemogram, electrocardiograms etc). The results of this research support the existence of a trichinellosis of acute phase (primary) and of a trichinellosis of chronic phase (secondary).

**78. Newborn with trichinellosis.** G. Enache<sup>\*</sup> and D. Panaitescu<sup>\*\*</sup>, <sup>\*\*</sup>The Clinical Hospital for Infectious and Tropical Diseases V Babes and <sup>\*\*</sup>The Cantacuzino Institute, Bucharest

This is the case of a male newborn, 10 months old, checked into The Clinical Hospital for Infectious and Tropical Diseases "Dr. Victor Babes" in March 2004, for whom the epidemiological and laboratory investigations, as well as, in a lesser measure, the clinical data, pointed to a diagnosis of trichinellosis. The epidemiological data revealed the fact that his father and his sister (6 years and 6 months old) had eaten pork and had been diagnosed with trichinellosis (also confirmed by serum analyses). The newborn (claimed his mother) had "taken" some of the meat from the plate. The newborn was admitted because of an erythematomaculo-papuloasa eruption, pluriginoasa, with alergodermatitis aspect. Laboratory investigations showed: number of leucocytes – 25.000/m<sup>3</sup>, with 30% eosinophils; TGP-184 u/l (the markers for acute viral hepatitis were negative); LHD – 499 u/l, and the serology for trichinellosis was negative. Under treatment with mebendazol – 100ml/day – 7 days, the response was favorable. 8 days after release, the state of the newborn was good, and the laboratory investigations revealed NL 15.100/mm<sup>3</sup>, and the eosinophils decreased to 10.2%. The newborn, as all the other members of the family diagnosed with trichinellosis are looked after at their polyclinic for a period of 2 years, in order to check the development of the disease after the acute phase, and to monitor the appearance of a secondary form of trichinellosis.



79. **Trichinellosis and diabetes.** G. Enache\* and D. Panaitescu\*\*. \*The Clinical Hospital for Infectious and Tropical Diseases V Babes and \*\*The Cantacuzino Institute, Bucharest

A particular complication in the development of trichinellosis is diabetes. It is thought that trichinellosis is not found in diabetes patients because the migration of the larvae is blocked. In our research, we have encountered 7 cases of diabetes in 123 patients with severe illness (5.69%). In the 7 cases, 6 were known at admission and one was discovered during the course of trichinellosis. In one of these cases we have noticed a rise of glycemia during the course of trichinellosis. Among the 7 cases of diabetes associated with trichinellosis, one has died, and 4 have answered the invitation of presenting themselves for clinical and laboratory check-up, performed 6-8 years after release from hospital. The glycemia levels remained high in 3 cases (between 180 and 254 mg/dl). In one of these cases, previous testing detected 415 mg/dl. The conclusion is that hyperglycemia encountered in these cases did not influence (either positively or negatively) the clinical course of trichinellosis. Even more, after longer intervals of time, the patients still presented clinical signs of trichinellosis. It would appear, thus, that the relationship of diabetes and trichinellosis is not consistent with the data from the specialized literature.

80. **An algorithm for diagnosing an acute *Trichinella* infection.** J. Dupouy-Camet<sup>1</sup> and F. Bruschi<sup>2</sup>. <sup>1</sup>National Reference Center on *Trichinella*, Parasitology Department, Hôpital Cochin, Université R. Descartes, 27 Fbg St Jacques, 75014 Paris, France <sup>2</sup>Department of Experimental Pathology, BMIE, University of Pisa, via Roma 55, 56126 Pisa, Italy

The clinical diagnosis of trichinellosis is difficult because there are no pathognomonic signs or symptoms but should be done asap as a medical treatment (e.g. albendazole and corticosteroids) is required to prevent severe complications. The following algorithm can be helpful for diagnosis but still needs further discussion and evaluation.

Group A	Group B	Group C	Group D
Fever	Neurological signs Cardiological signs	Eosinophilia (>1,000/mm <sup>3</sup> ) and/or increased total IgE levels	Positive serology (with a highly specific test)
Facial and/or eyelid oedema	Conjunctivitis Subungueal haemorrhages	Increased levels of muscular enzymes	Sero-conversion
Myalgia	Cutaneous rash Diarrhoea		Positive muscular biopsy
The diagnosis is: Very unlikely if one A or one B or one C ; Suspected if one A or two B and one C ; Probable if three A and one C; Highly probable if three A and two C; Confirmed if three A, two C, and one D or any of groups A or B and one C and one D			

**81. Persistence of reactivity against the 45 kDa glycoprotein (45 gp) in late trichinellosis patients.** F. Bruschi<sup>1</sup>, M.T. Locci<sup>1</sup>, W. Cabaj<sup>3</sup>, B. Moskwa<sup>3</sup>, B. Castagna<sup>1</sup>, W. Kociecka and M. Masetti<sup>2</sup>, Departments of <sup>1</sup>Experimental Pathology, M.B.I.E., and of <sup>2</sup>Ethology, Ecology and Evolution, Università di Pisa, Pisa, Italy; <sup>3</sup>W. Stefanski Institute of Parasitology, Polish Academy of Sciences, Warszawa, Poland

During the years, opinions of clinicians on the existence of the so-called chronic trichinellosis or late sequelae of infection have been controversial. However, persistence of humoral immune response against *Trichinella* in these late patients has been confirmed also using specific tests such as the competitive inhibition assay (CIA). We evaluated sera from late trichinellosis patients (2-8 years from infection), for their reactivity against *T. spiralis* antigens. The following tests were carried out: i) an indirect immunofluorescence assay (IFA), performed on muscle sections from 30 day synchronously infected mice, i.e. injected with *T. spiralis* newborn larvae; ii) CIA; iii) EIA, employing a synthetic antigen represented by  $\alpha$ -tyvelose conjugated to bovine serum albumin (BSA-Ag); iv) western blot (WB) with both an "in house" kit and a commercial one. The results of IFA observed by confocal laser microscopy - which resulted particularly helpful in antigen localization - showed that sera evaluated reacted against both surface and internal structures of L<sub>1</sub> larvae but with a different extent correlated to the titers observed with quantitative serological tests such as CIA. EIA employing the BSA-Ag has shown that all sera tested resulted positive for the presence of specific antibodies against  $\alpha$ -tyvelose. WB showed that all sera were reactive with the 45 gp. All these data suggest that the reactivity against the  $\alpha$ -tyvelosilated 45 gp persist during infection, even in very late periods.

**82. Re-evaluation after 15 years of patients involved in a trichinellosis outbreak caused by *Trichinella britovi*.** D. Piergili-Fioretti<sup>1</sup>, B. Castagna<sup>2</sup>, O. Vittori<sup>3</sup>, D. Frondizi<sup>3</sup>, R. F. Frongillo<sup>4</sup> and F. Bruschi<sup>2</sup> Dept. of <sup>1</sup>Veterinary Biopathological Science, and <sup>4</sup>Clinic of Infectious Diseases, Terni, Università di Perugia, Perugia, Italy; <sup>2</sup>Dept. of Experimental Pathology, M.B.I.E., Università di Pisa, Pisa, Italy; <sup>3</sup>Neurophysiopathology Unit, Ospedale Santa Maria, Terni, Italy

We had the opportunity to re-evaluate 13 out of 48 subjects involved in a trichinellosis outbreak occurred in Central Italy (Umbria Region) in 1988, caused by the consumption of sausages made with raw boar meat infected with *T. britovi*. During the outbreak, 28 out of 48 serologically positive (IFA titers between 1:16 and 1:1,024) subjects were asymptomatic, whereas 20 of them presented clinical signs such as fever (100%), myalgias (100%), periorbital oedema and conjunctivitis (85%), 3 patients were hospitalised because of severe clinical signs and 2 of them were treated with mebendazole and corticosteroids. Of the 13 re-evaluated patients, no one presented clinical signs, only 3 had still increased CPK or LDH serum levels, and some of them presented electromyographic changes. We evaluated their sera for reactivity against *Trichinella britovi* antigens, with EIA using both E/S antigen and a synthetic antigen represented by  $\alpha$ -tyvelose conjugated to bovine serum albumin (BSA-Ag) and with western blot (WB), carried out with a commercial kit. The results obtained showed that in both EIA tests only 2 sera resulted still positive, whereas in WB all sera reacted to several *Trichinella* antigens, in particular to the  $\alpha$ -tyvelosilated 45 kDa. glycoprotein. The results show that also *T. britovi*, considered less pathogenic than other *Trichinella* species, is responsible for late sequelae.

83. **Heart specific antigens recognized by trichinellosis patient sera.** F. Bongiorni<sup>2</sup>, S. Tommasi<sup>2</sup>, S. Mazzoni<sup>1</sup>, P. Migliorini<sup>2</sup> and <sup>1</sup>F. Bruschi, Depts. of <sup>1</sup>Experimental Pathology, M.B.I.E. and of <sup>2</sup>Internal Medicine, Università di Pisa, Pisa, Italy

Heart can be seriously affected in human trichinellosis, and cardiac involvement can be cause of death. The pathogenesis of heart involvement has not yet been clarified and the mechanical damage induced by migrating larvae has been considered the major cause. Experimental infections in rats have shown, however, that when parasite presence is no more detectable in the myocardium, even using molecular methods, the heart function is still reduced and immunopathological changes such as eosinophil and mast cell infiltration and immune-complex deposition occur, finally leading to a dilated cardiomyopathy. Sera from trichinellosis patients, were tested by immunoblot on extract of rat ventricle and on liver, spleen, and skeletal muscle extracts as control. Patients sera recognized several antigens that were not bound by normal sera. On rat ventricle 5/21 sera bound an antigen of 65.9 kDa, 4/21 sera bound an antigen of 76.45 kDa. These antigens are tissue specific since are not present in liver, spleen and skeletal muscle extracts. Antibodies recognizing heart specific antigens have been found also in patients affected by viral myocarditis or primary dilated cardiomyopathy. However, the role of antibodies in inducing cardiac dysfunction and the mechanisms eliciting their production are still unknown. Molecular mimicry between antigens shared by host tissues and infectious agents is a possible mechanism. Studies are in progress to shed more light on these new pathogenetic mechanisms of the cardiomyopathy occurring during human trichinellosis.

84. **Rehabilitation of Trichinellae.** V. A. Britov, E. A. Nivin and I. N. Lukashkova, Primorsky Research Veterinary Station, Russia, Vladivostok, mail-box 123, 690002

The myth about an extremely tortious act of trichinellae which has been exaggerated since the Bible times does not correspond with the reality. For food and home trichinellae “pay” their master in the induction of cellular immunity. According to F. Bennett’s theory cellular immunity possesses the quality of being nonspecific and serves the basis of the most ancient defend of people and animals against everything alien. Harm and use are philosophical categories, they co-exist in one unity. In conformity to trichinellae we may speculate in such a way: if there is harm in them there must be use as well. It is easily proved experimentally. With a purpose of cellular immunity induction we used a special line of trichinellae derived by means of selection. The medication was taken together with the authors by 2,000 patients-volunteers who had not got a medicinal effect at official medical institutions. The effectiveness of the medication turned out to be very high. A number of works have been published about the results. Now we have come to the belief that for a man and warm-blooded animals trichinellae are symbionts rather than parasites. With the invention of antitrichinellosis remedies there appeared a real chance of using trichinellae for prophylaxis and medical treatment for diseases of a human immune system – the main reason of great number of diseases. Thus, in the arsenal of biotherapy a new highly effective remedy has appeared – the medication from trichinellae.

**85. Evaluation of albendazole in intestinal and muscular phase infection by *Trichinella spiralis* in a murine model.** Alejandra Moreno-García<sup>1</sup>, Gabriela Reveles-Hernández<sup>1</sup>, Isabel Chávez- Ruvalcaba<sup>1</sup>, Jesús Muñoz-Escobedo<sup>2</sup> <sup>1</sup>Unidad Académica de Biología Experimental, <sup>2</sup>Unidad Académica de Odontología, Universidad Autónoma de Zacatecas., Apartado Postal 12, Guadalupe, Zac. México. CP. 98600

Ten species of *Trichinella* had been described in México. The pig is the primary source of infection. Even though a definitive treatment is not established, good results have been obtained in the intestinal phase with benzimidazoles. Treatment of muscle infection is yet in the experimental stage. Our objective was to evaluate 3 treatment regimens of albendazole on the intestinal and muscular phase of trichinellosis in a rodent model. Long Evans rats separated in 4 groups of 12. The first group was infected with 500 infective larvae (IL) and were treated for 1,3,5 and 10 days with albendazole (15 mg/kg daily, p.o.). The second group was infected with 500 IL and at the seventh day post infection, were treated for 1,3,5 and 10 days. Third group was infected with 500 IL and 14 day post infection, were treated for 1,3,5 and 10 days. The fourth group was infected with 500 IL and ten weeks post infection, were treated the 1,3,5 and 10 days. Rats were bled pre- and post-infection to characterize the immune response by Western blot (WB). Groups 1, 2 and 3 were sacrificed 8 weeks post infection. We evaluated by direct techniques (tissue compression, artificial digestion and H and E) the parasite burden. In the first group we found some parasites with one day of treatment. In groups 2 and 3 we found more parasites, but those were decreasing with increasing days of the treatment. In group 4 we found parasites in the 1, 3 and 5 days of treatment, but after 10 days, *T. spiralis* were not viable. Albendazole is effective in intestinal and muscular phase, but the timing of treatment is critical. We recommend ten days.

**86. Evaluation of three anthelmintics on intestinal and muscular phase infection of *Trichinella spiralis* in the pig model.** Isabel Chavez Ruvalcaba<sup>1</sup>, Gabriela Reveles-Hernández<sup>1</sup>, Sergio Saldivar- Elias<sup>1</sup>, Jesús Muñoz-Escobedo<sup>2</sup> Alejandra Moreno-García<sup>1</sup>. <sup>1</sup>Unidad Académica de Biología Experimental. Universidad Autónoma de Zacatecas. México. Apartado Postal 12. Guadalupe Zacatecas. México. CP. 98600, <sup>2</sup>Unidad Académica de Odontología. Universidad Autónoma de Zacatecas

Trichinellosis is a parasitic disease that affects Zacatecas people. Pork is a source of infection. We evaluated the effect of 3 anthelmintic drugs on infection caused by *Trichinella spiralis* in pigs. York race pigs (18 weeks old), were divided in 9 group: 1) 2 control pigs uninfected, 2) 2 control pigs infected with *Trichinella spiralis*, 3) 2 infected pigs treated with albendazole 400 mg/per day / 3 days during the intestinal phase, 4) 2 infected pigs treated with ivermectin (200 µgr/Kg), one dose during the intestinal phase, 5) 2 infected pigs treated with nitazoxamid 7.5 mg/Kg /per day for 3 days during the intestinal phase, 6) 2 control pigs infected with *T. spiralis*, 7) 2 infected pigs treated with albendazole 400 mg/per day during muscular phase, 8) 2 infected pigs treated with ivermectina 200 µgr/Kg, one dose during muscular phase, 9) 2 infected pigs treated with nitazoxamid 7.5 mg/Kg /per day during the muscular phase. The dose of infection was 10 *T.spiralis* by gram body weight. The immune response was assessed by Western blot, the parasite burden was evaluated by tissues compression, artificial digestion, viability by trypan blue and and Hand E staining of muscle and infection of mice. The most effective drug was albendazole. In the muscular phase, nurse cells suffered changes, larvae were not viability or infectious. Ivermectin and the nitazoxamid were less effective.

87. **Effect of albendazole in pregnant rats infected with *Trichinella spiralis*.** Gabriela Reveles-Herández<sup>1</sup>, Sergio Saldivar-Elias<sup>1</sup>, Alejandra Moreno-García<sup>1</sup>, Jesús Muñoz- Escobedo<sup>2</sup>, <sup>1</sup>Unidad Académica de Biología Experimental. Universidad Autónoma de Zacatecas. México. Apartado Postal 12. Guadalupe Zacatecas. México. CP. 98600, <sup>2</sup>Unidad Académica de Odontología. Universidad Autónoma de Zacatecas

Background: Albendazole is teratogenic and embryo toxic in rats and rabbits but the precise effects at different dosages are unknown. In the last 10 years use of this medicine has increased. There is the need to evaluate the effect of the drug during gestation. Objective: Evaluate the effect of albendazole in pregnant rats infected with *Trichinella spiralis*. Methods and Materials: 50 rats (10 weeks) of reproductive age were divided in 10 groups of 4 animals each. Group 1. Healthy control, 2.- Pregnant control, 3.- Pregnant rats with one day of treatment of albendazole, 4.- Pregnant rats with three day treatment with albendazole, 5.- Pregnant rats with ten days treatment with albendazole, 7.- Pregnant rats, infected with 500 *T. spiralis* and 1 day of treatment, 8.- Pregnant rats infected with *T. spiralis* and 3 day of treatment. 9.- Pregnant rats infected with *T. spiralis* and 5 days of treatment with albendazole, 10.- Pregnant rats infected with *T. spiralis* and 10 days treatment with albendazole. Litters were evaluated at delivery for either size, pup morphology, reproductive capacity (at 3 months) immune response and parasite burden. Results: Pregnant controls bore 10-12 pups. Pregnant rats treated for one day were normal, groups treated with 3, 5 and 10 days suffered multiple deformities. 1 day of treatment, did not affect parasite burdens while 3, 5 and 10 days of similar treatment prevented parasite development. Conclusion: Albendazole is a medicine that mustn't be used in pregnant females for more that 3 days. Albendazole is effective against *T. spiralis* but requires a minimum treatment of 10 days.

88. **An immuno-polymerase chain reaction assay for circulating antigens in trichinellosis.** L.Hui, X.Bianli\*, Z.Xudong and D.Yan, Henan Provincial Center for Disease Control and Prevention, No.47, Weiwu Road, Zhengzhou, Henan, P.R. China, 450003

A highly sensitive immuno-PCR assay for detecting circulating antigens in trichinellosis was set up, which is developed from a sandwich ELISA and PCR. Antigens were purified from muscle larvae of *T. spiralis*. Myeloma cell were fused with splenocytes of mouse immunized with *T. spiralis* antigens. Selection of antibody-secreting hybridomas cell was done by indirect ELISA. Monoclonal antibody (F4C6) against *T. spiralis* ES antigen was obtained, which was used as indicator antibody, and rabbit homologous antibodies against *T. spiralis* was used as capturing antibodies. Plasmid Bluescribe II KS(+) was amplified by PCR with Biotin labeled M13-20 primer and Biotin labeled DNA was obtained. The second antibody and DNA were labeled by Biotin respectively, and they both were linked up by avidin. Just the right amount of avidine was 100ng/ml and the Bio-DNA was 10pg/ml. The assay had two steps, first the circulating antigens was captured by monoclonal antibody through sandwich ELISA, and second step the DNA linked by monoclonal antibody was amplified by PCR. The sensitive of Immuno-PCR assay for detecting circulating antigens in trichinellosis was compared with ELISA. The measuring arrange for detecting circulating antigens in trichinellosis was from 5  $\mu$ g/ml to 0.05  $\mu$ g/ml for ELISA and from 50pg/l to 0.05pg/l for Immuno-PCR assay. The Immuno-PCR assay is firstly applied in detecting circulating antigens in trichinellosis and is highly sensitive.

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**89. Detection of anti-*Trichinella* antibodies in chronically infected horses by IFA and Western blot, but not by ELISA.** Lj. Sofronic Milosavljevic<sup>1</sup>, N. Ilic<sup>1</sup>, M. Djordjevic<sup>2</sup>, M.Savic<sup>2</sup>, A. Gruden-Movsesijan<sup>1</sup>, K. Cuperlovic<sup>2</sup>, K.D. Murrell<sup>3</sup>, INEP<sup>1</sup>, IMHT<sup>2</sup>, 11080 Belgrade, Serbia and Montenegro; Danish Center for Experimental Parasitology<sup>3</sup>, Royal Veterinary and Agricultural University, Copenhagen, Denmark

The use of an indirect method to detect *Trichinella* infection in horses is still a problem because of the lack of specificity and sensitivity of current serology methods. The dynamics of anti-*Trichinella* IgG production and circulating ES antigen presence were investigated in three horses that were experimentally infected by feeding infected meat with very low level *T.spiralis* muscle larvae. Serum samples were collected in 2 week intervals for serology testing. Horses were slaughtered and necropsied at 32 weeks post infection (p.i.). All 3 animals harbored a low worm burden in their muscles. Detection of specific antibody was performed by IFA, ELISA (using either the ES or synthetic Tyvelose-BSA antigens) and Western blot (ES antigen). The presence of ES antigen in sera of infected horses was followed by Dot blot. Circulating IgG was detected up to 32<sup>nd</sup> week p.i. by IFA and Western blot, but not by ELISA. ELISA test, disregarding applied antigen, detected anti-*Trichinella* IgG for only a short period of time. Western blot revealed presence of anti-*Trichinella* IgG by the appearance of a specific band triad pattern (45,49,53 kDa). Based on this finding, which agrees with our unpublished results on band triad existence in other species infected with *T.spiralis* (human, swine, dog), we believe that the monitoring of those bands is a useful method for antibody detection in different species. The presence of the ES antigen in the circulation was observed from the 4<sup>th</sup> week p.i. up to the 32<sup>nd</sup> week p.i. For the investigations of *Trichinella* prevalence in horses we suggest examining simultaneously for the presence of ES Ag and specific antibody reflected by the appearance of specific band triad in Western blots.

**90. Trichinellosis in Argentina: an historical review** M Ribicich, HR Gamble, J Bolpe, A Rosa, A Franco. Parasitology and Parasitic Diseases. Facultad de Ciencias Veterinarias. Universidad de Buenos Aires, Chorroarín 280. (C1427CWO) Ciudad de Buenos Aires, Argentina

In Argentina, *Trichinella* infection in pigs is endemic; it is detected, frequently, through diagnosis and notification from human cases. The first report of human trichinellosis in Argentina was from 1898 in Buenos Aires, and, as in other countries of the world, it was diagnosed as typhoid fever. The number of human cases increased from 908, between 1971 and 1981, to 6919, between 1990 and 2002. In pigs slaughtered in official establishments, the prevalence of *Trichinella* infection was 0.46% in 1914 and 0.01-0.03% during the period 1990-2004. *T. spiralis*, is typically found in the domestic cycle that includes pigs, humans and rodents. *Trichinella* spp. from a sylvatic cycle have also caused human outbreaks resulting from the consumption of meat from puma, armadillo and wild boar. European migration to Argentina (principally Spanish and Italian) during the first years of the 20<sup>th</sup> century brought the tradition of preparing and eating raw sausages, increasing the risk of human exposure to *Trichinella*. Detection in pigs was initially made at slaughter by compression of muscle tissue. Sanitary officials, in 1944, increased control methods, using trichinoscopy until 1996, then artificial digestion for preventing human trichinellosis in Argentina.

91. **Epidemiological investigation for the identification of a trichinellosis focus.** R. Olariu<sup>1</sup>, L. Negrutiu<sup>1</sup>, G. Darabus<sup>2</sup>, I. Iacobiciu<sup>1</sup>, A. Koreck<sup>3</sup>, and I. Marincu<sup>1</sup>, University of Medicine and Pharmacy Timisoara, Romania<sup>1</sup>, Faculty of Veterinary Medicine Timisoara, Romania<sup>2</sup>, Faculty of Medicine, University of Szeged, Hungary<sup>3</sup>

Epidemiological investigations represent a complex modality of study and research of those cases which determine the appearance and the dissemination of a parasitic disease. We have studied the importance and the efficiency of the epidemiological investigation in the identification of the trichinellosis in a gipsy collectivity. Epidemiological investigations, trichinoscopy, clinical diagnosis and laboratory tests were used as methods in this study. Diagnosis was made on the clinical symptoms: headache, weakness, muscle pain, diarrhea, edema-chiefly orbital, and fever. Laboratory investigations confirmed leukocytosis and high values of eosinophils. As a feature, we mention the poor socio-economical conditions of the patients and the low and precarious level of sanitary conditions. The epidemiological investigations allowed us to conclude that the disease appeared after consuming pork meat proceeded from a garbage pit situated in a rural area of the Timis County, Romania. *Trichinella spiralis* was identified from the remaining pork meat by trichinoscopy. We have identified 28 de patients with trichinellosis, who were isolated and treated in the clinic of infectious disease from Timisoara, Romania. We have noted 8 asymptomatic cases, 14 patients with moderate forms of disease, and 6 severe forms of trichinellosis. It was established the contamination source, the transmission way and the social economical conditions of the patients. Prevention and control measures were taken including the isolation and the therapy of the patients. We conclude that epidemiological investigations may be an efficient method for the identification of the infection source, isolation, prevention and control of this parasitosis.

92. **Experimental studies in SPF pigs on *Trichinella* detection in different diagnostic matrices.** K. Noeckler<sup>1</sup>, F. J. Serrano Aguilera<sup>2</sup>, Federal Institute for Risk Assessment (BfR)<sup>1</sup>, Diedersdorfer Weg 1, 12277 Berlin, Germany, University of Extramadura<sup>2</sup>, Avda de la Universidad s/n, 10071 Cáceres, Spain

TRICHIPORSE - a research project of the EU (QLRT-2000-01156) is focussed on direct and indirect methods for trichinellosis diagnosis in food animals. One topic refers to diagnostic tools which can be applied for certification of *Trichinella*-free pig farms. In 4 trials 3 SPF and Iberian pigs per group were inoculated with 200, 1,000 and 20,000 muscle larvae of *T. spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis*, respectively. Samples from 9 muscles were examined for larvae by magnetic stirrer method. Blood samples were taken at days -4 and -1 prior and 5, 10, 15, 20, 25, 30, 40, 50 and 60 post infection. Serum was tested for anti-*Trichinella*-IgG by means of E/S-ELISA and antibody content in serum and meat juice of diaphragm and tongue was examined at dilutions from 1:10 to 1:1280 and 1:1 to 128, respectively. Larval recovery rate (LpG) in SPF/Iberian pigs corresponded with infection dose in the following order: *T. spiralis* mean LpG/group: 2.8/3.0, 15.9/43.1, 416.8/538.8; *T. britovi*: 0.01/1.82, 0.07/0.98, 3.53/123.1; *T. pseudospiralis*: 0.002/0.058, 0.043/2.1, 9.66/98.9 and *T. nativa*: 0/0.001, 0.006/0.002, 0.02/0.1. In most cases tongue, diaphragm and masseter were identified as predilection sites. In ELISA time of seroconversion correlated with infection dose. In pigs infected with 20,000 larvae specific IgG was detected at days 25 to 40 p.i. and was delayed or not detectable in pigs infected with 1,000 and 200 larvae. With regard to content of anti-*Trichinella*-IgG in serum compared to meat juice there was a good correspondence when dilution ratio was 100:10. Laboratory results indicate suitability of blood serum and meat juice for checking *Trichinella* status in living pigs and slaughter carcass, respectively. Further investigations on test validation are planned in pig farms and slaughterhouses.

93. **Epidemiology of equine trichinellosis: the risk from animal protein feeding practices.** K.D.Murrell<sup>1</sup>, M. Djordjevic<sup>2</sup>, K. Cuperlovic<sup>2</sup>, Lj. Sofronic<sup>3</sup>, M. Savic<sup>2</sup>, M. Djordjevic<sup>4</sup>, S. Damjanovic<sup>5</sup>, Danish Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, Denmark<sup>1</sup>, Institute for Meat technology and Hygiene, Belgrade<sup>2</sup>, INEP, Zemun<sup>3</sup>, Veterinary Centre, Mladenovac<sup>4</sup>, Slaughterhouse “Damjanovic”, Mladenovac, Serbia and Montenegro

A discovery in 2002 of a *Trichinella spiralis*- infected horse in Serbia offered an opportunity to conduct epidemiological studies on how horses, considered herbivores, acquire a meatborne parasite.. Traceback of the infected horse to a farm owner was carried out and investigations on the farm led to the conclusion that the owner had fed the horse animal products and kitchen waste in order to condition the horse prior to sale. Based on interviews of 31 horse breeders and dealers in the region, it was confirmed that the feeding of animal protein products to horses was a common practice, especially prior to sale. Further, it was alleged that many horses, particularly those in poor nutritional condition would readily consume meat. To test this, we conducted a series of trials involving the experimental feeding of 219 horses meat in various forms. Overall, 32% of the horses readily consumed meat. To confirm that *Trichinella* would be transmitted to horses fed infected meat under normal farm conditions, 3 horses were offered infected ground pork balls. All three became infected, and remained so until necropsy 32 weeks later. All were still positive by indirect IFA testing, but not by ELISA using an excretory-secretory antigens. These results indicate that further study is needed on the nature of the antigen(s) used for potential serological monitoring and surveillance of horse trichinellosis, especially the importance of antigenic diversity, which is characteristic of the “antigen” used in the IFA.

94. ***Trichinella nativa* in experimentally infected seals.** C.M.O. Kapel<sup>1</sup>, L. Measures<sup>2</sup>, L. Moeller<sup>1</sup>, L. Forbes<sup>3</sup> and A. Gajadhar<sup>3</sup>, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark<sup>1</sup>, Fisheries and Oceans Canada, Mont-Joli, Quebec, Canada<sup>2</sup>, Centre for Animal Parasitology, Canadian Food Inspection Agency, Saskatoon, Saskatchewan, Canada<sup>3</sup>

A few studies from Arctic have found *Trichinella* sp. in seals, but the zoonotic importance of infected seal meat is unknown. Meat of walrus and polar bears is frequently infected with *Trichinella nativa* and represents a significant health risk for Inuit populations, but even where these meats are not consumed the human seroprevalence is high and seal meat is suspected as the source. Four female grey seals, 12 weeks of age, were inoculated with *Trichinella nativa*. An isolate from a polar bear, which has been maintained in mice, was passed through foxes prior to inoculation of the seals. Two seals received 50,000 larvae (1,000 l/kg body weight), the remaining two 5,000 larvae (100 l/kg). Blood samples were collected weekly. Two seals were sacrificed 5 wpi, the remaining 10 wpi. To evaluate the freeze resistance of *T. nativa* in seals, muscle tissue was stored at +5, -5, and -18 C. After 1, 4, and 8 weeks the larvae in the muscle tissue was released and inoculated into mice. Infection established in all four seals. A marked dose-response correlation was found and seals killed 10 wpi had higher larval burdens. Diaphragm was found to be a predilection site. All seals sero-converted 3-4 wpi, but antibody levels increased up to 8 wpi. Muscle larvae were able to survive for 8 weeks at temperatures ranging from +5 to -18. The study demonstrated that seals are very susceptible to infection with *Trichinella* and that muscle larvae are infective even after deep freezing. Thus, even though the prevalence among seals is very low, infected animals might constitute a significant zoonotic threat.



**95. Epidemiology: frequency of *T. spiralis* in horses from two slaughter house (Municipal and rural) in the State of Mexico.** E. Jimenez-Cardoso<sup>1</sup>, M.L. Caballero García<sup>1</sup>, E. Trejo-Hernández, G. Uribe-Gutiérrez, F.R. Gay-Jiménez<sup>2</sup>. <sup>1</sup>Parasitology Research Laboratory, Hospital Infantil de Mexico Federico Gómez. <sup>2</sup>Department of Agricultural and Animal Production, UAM Xochimilco Mexico

The aim of this investigation was to determine the frequency of *Trichinella spiralis* in horses from two slaughter houses: Municipal Rural in the State of Mexico and a rural horse slaughter house. We study two groups; one of 70 horses from a municipal slaughter house in San Vicente Chicoloapan and the second, 80 horses from a rural slaughter house in Angel Peralta of the Nevada Mountain, both from the State of Mexico. Ten grams of muscle, diaphragm and tongue as well as 10 ml of blood were obtained from each animal. Macroscopic identification of *Trichinella* was carried out by trichinoscopy and artificial digestion and molecular identification was done by PCR using pPRA primers on tissues and blood (Dick et al J. Parasitology 1992). The frequency of infection using artificial digestion in the municipal and rural slaughter houses was 1.36% and 1.2% respectively. Identification of the *T. spiralis* using PCR in muscles and blood samples, was positive in 4.2% and 3.75% in the municipal and rural slaughter houses respectively. The methods of direct diagnosis didn't allow identification of infected animals. Molecular analysis by PCR was positive for *Trichinella* in both groups. These results suggest that the last one is a more sensitive and specific method of detecting infected animals. Its use will allow to design control methods of animal transmission to humans.

**96. The epidemiology of trichinosis in the Jiu Valley in the 1987-2003 period (Summary).** Debora Cristea<sup>1</sup>, Eugenia Cristea, The Medicine and Pharmacy University Carol Davila, Bucharest, student the VI-th year, Romania; <sup>2</sup>The Individual Medical Cabinet, general doctor, N.Titulescu street, bl.T3, 336200- Vulcan, Romania

Epidemiological studies were carried out in towns of the Jiu Valley, the most important trichinosis focus from Romania in the 1987-2003 period. The first Trichinosis focus in men in the Jiu Valley appeared in Aninoasa town in 1965. In 1989, 191 ill persons were reported, and in 1991 a peak of 570 cases of human Trichinosis (323.86 ill persons per 100.000 inhabitants) were reported – a value of 22.6%. This year (1991) the Trichinosis in men in the Jiu Valley accounted for 33.3% of all the cases recorded in Romania. In the 1992 year, the number of trichinosis cases decreased to 435. In 1993, 305 people became ill and in 1994, 34. Due to prophylactic measures which were undertaken in 1995, the number of ill persons was 42, and in 2001 and 2003 there were no cases of Trichinosis in men. In the 1997-2003 period, the number of Trichinosis cases in men was of 2524.

97. **The epizootology of trichinosis in the Jiu Valley in the 1988-2003 period (Summary).** Gh. Cristea<sup>1</sup>, Eugenia Cristea<sup>2</sup>, Debora Cristea, The Veterinary District Vulcan, veterinary, N.Titulescu street, No.42,336200-Vulcan, Romania;<sup>2</sup>The I. M. C.-general doctor, Romania; <sup>3</sup>The U.M.F. Carol Davila, Bucharest, the VI-th year student, Romania

The epizootological studies, were effected in the Jiu Valley. As a focus with sanitary implications, Trichinosis appears in the 1988 year, when from 233 433 examined pigs in Vulcan and Petrosani cities, 12 pigs were found infested (2.77%). During the years, Trichinosis extends in the Jiu Valley reaching a value of 42 infested pigs from 1247 examined ones (3.36%) in the 1989 year. The ill animals' number reaches the high tide in the 1992 year, when were declared infested 1281 pigs from 16840 examined animals, representing a value of 7.6%, the great incidence was in Vulcan city – 15.4% (Vulcan is Trichinosis' pole in Romania). Due to the measures which have been undertaken to stop this parasitical aval, the Trichinosis' incidence in pigs decreases every year reaching a value of 16 infested pigs from a number of 14288 examined ones in the Jiu Valley (0.11%) in the 2003. in the 1988-2003 period in the Jiu Valley's localities, were killed and examined trichineloscopically 215936 pigs and were found infested 3928, which means 1.8%. The Trichinella larvae were found in other animals species, they developed and continued to pollute the environment. This, the rats and the dogs were really parasitical storages. Examining 492 rats cadavers, we found 204 infested, meaning a value of 41.5%. Through trichineloscopical examination, we found 54 infested dogs from 148 dogs cadavers examined. We found examining trichineloscopically 7 boars infested (21.2%) from 33 examined, 5 Trichinella larvae infested foxes among 16 examined animals (31.25%), among 38 mice 4 were infested (10.52%).

98. ***Trichinella pseudospiralis* from a wild pig in Texas, USA.** H. R. Gamble<sup>1</sup>, E. Pozio<sup>2</sup>, J.R. Lichtenfels<sup>1</sup> and D. S. Zarlenga<sup>1</sup>, U.S. Department of Agriculture, Agricultural Research Service, Beltsville.,MD<sup>1</sup> 20705 and Department of Infectious, Parasitic and Immunomediated Diseases, Instituto Superiore di Sanità, Rome, Italy<sup>2</sup>

Wild boar intended for export are inspected in the U.S. using artificial digestion methods. In December 2001, the routine inspection of a wild pure Russian boar (*Sus scrofa*) harvested near Newcastle, Texas (33N12, 98W44) revealed the presence of *Trichinella* ssp. larvae. A total of 3300 larvae were recovered from 23 grams of host muscle for a worm burden of 143.5 larvae per gram of diaphragm tissue. Biological, morphological and genetic analyses demonstrated the parasite to be *T. pseudospiralis*. Worms passed in Swiss Webster mice failed to develop a capsule 6 month after infection. Measurements of muscle larvae recovered from mice were as follows: males – 672 µm and females – 682 µm. PCR analysis confirmed the identity of this isolate as *T. pseudospiralis*. This is the second report of *T. pseudospiralis* in the United States; an isolate of this species was previously obtained from a culture in Alabama. Analyses of three genes, cytochrome oxidase subunit I, large subunit RNA, and expansion segment five, revealed that this isolate is very similar to the isolate obtained from Alabama, both belonging to the Nearctic population of *T. pseudospiralis*. The presence of this parasite in a food animal species emphasizes the importance of routine inspection using artificial digestion methods, which can detect non-capsule forming species of *Trichinella*.

99. **Epidemiological investigation of *Trichinella* spp. in wild boars in Croatia.** S. Bosnić<sup>1</sup>, A. Marinculić<sup>2</sup>, M. Benić<sup>1</sup>, R. Beck<sup>2</sup>, Veterinary Institute<sup>2</sup>, Savska 143, 10 000 Zagreb, Croatia; Department of Parasitology<sup>1</sup>, Veterinary Faculty, University of Zagreb, Heinzelova 55, 10 000 Zagreb, Croatia

The prevalence study of *Trichinella* spp. in wild boars was conducted as a part of National Prevalence Study of Trichinellosis in endemic and nonendemic areas of Croatia. The artificial digestion of muscle samples from diaphragm and front legs of 132 wild boars larvae revealed the prevalence rate of 3.03% (4 animals). Sera and muscle juice samples from all animals were also examined by ELISA using the ordinary excretory-secretory antigen. Among 132 sera antibodies against ES antigen were found in 36.06 (48 sera) samples. Only 9.09% (12 samples) of muscle juice samples were found positive. We also compared the seroprevalence among animals from endemic and non endemic areas of the country. In endemic areas 35 (72.91%) animals were found with antibodies. The analysis between sexes clearly shows that the seroprevalence was higher in females (44.3%) in the comparison to males (27.4%,  $P < 0.05$ ). No differences between the age were found.

100. **Effects of social-economic factors on epidemic process at *Trichinella spiralis* infection in Russia.** A.S. Bessonov, K.I. Skryabin Institute of Helminthology, Moscow, Russia

The social-economic upheavals in Russia taken place at the end of the last century reflected on all sides of life of country including prevalence of diseases in general and *Trichinella spiralis* infection in particular. The aim of the work was to monitor the epidemic situation over the last 40 years covering the period of blossoming of large scale state animal husbandry and its degradation. One performed analysis and generalization of the statistical data on prevalence of *T. spiralis* infection. The reduction of the rate of *T. spiralis* infection in swine took place over the period of 1962-1971 from 0.011% to 0.00313% or by 3.51 times. The decrease of infection extensiveness over the period of 1971-1980 from 0.00313% to 0.00012% or by 26.08 times was much more impressive. However the number of infected swine sharply increased over the period of 1980-1992 from 0.00012% to 0.002% or by 16.7 times as while in 1992-2001 it continued to increase slowly from 0.002% to 0.003% or by 1.5 times. The mean rates of *T. spiralis* infection in swine over the period of 1992-2001 evidenced about the highest endemicity values in the Krasnodar Territory (169.6 cases) and the North Ossetia (151.8 cases). The Moscow Area (90.2 cases) and the Krasnoyarsk Territory (88.8 cases) comprised the second group on endemicity followed the third one – the Murmansk, Kaliningrad, Rostov, Vologda and Leningrad Areas with infection extensiveness in the range of 48.7 to 22.3 cases respectively. *T. spiralis* infection in humans predominated in the South (337 cases) and Siberia (350 cases) Federal Districts according to the data over the period of 2000-2001. In other Federal districts the rate of infection in human population was significantly lower (in the Central – 90, North-West – 46, Pryvolzhsk – 68, Ural – 77, Far-East – 156 cases respectively). The high rate of infection in humans in a several Federal districts (South, Siberia, Far-East) to a great extent in associated with consumption of wild animals meat obtained on hunting (wild boars, bears) and exotic dishes from meat of dogs, foxes, badgers and other.

101. **Survey on porcine trichinellosis in Ecuador.** M.A. Chávez-Larrea<sup>1</sup>, P. Dorny<sup>2</sup>, L. Moeller<sup>3</sup>, W. Benítez-Ortiz<sup>1</sup>, M. Barrionuevo-Samaniego<sup>1</sup>, R. Rodríguez-Hidalgo<sup>1</sup>, J. Ron-Román<sup>1</sup>, F. Proaño-Pérez<sup>1</sup>, B. Victor<sup>2</sup>, J. Brandt<sup>2</sup>, C. Kapel<sup>3</sup>, J. de Borchgrave<sup>2</sup>, Centro Internacional de Zoonosis, CIZ, Universidad Central del Ecuador<sup>1</sup>, Institute of Tropical Medicine, ITM, Antwerp, Belgium<sup>2</sup>, Danish Centre for Experimental Parasitology, DCEP, Frederiksberg<sup>3</sup>

A survey on porcine trichinellosis was organised in Ecuador between 2000 and 2003. Blood samples were taken for serological analysis in slaughterhouses (study 1, N=2000; study 2, N=331) and in a village where pigs are free roaming (study 3). Muscle samples for parasitological examination were taken from pigs slaughtered in the abattoir (study 2) and from animals that showed a positive serology (study 3). Analysis of the sera by ELISA using excretory/secretory (E/S) antigen detected 7 positives out of 2000 (0.35%) (study 1) and 0 out of 331 pigs (study 2). Sero-positives were confirmed by ELISA using tyvelose antigen and by immunoblot. Thirty-seven (5.72%) village pigs tested positive in E/S ELISA in study 3. Trichinoscopy and artificial digestion failed to demonstrate the presence of muscle larvae in the pigs sampled in studies 2 and 3. The results of this survey suggest that *Trichinella* is present in Ecuador, however, prevalence and parasite burdens are likely to be low. Chances to detect trichinellosis are probably higher in traditional settings than in pigs presented at slaughterhouses.

102. **Trichinosis in Armenia.** A. Asatrian<sup>1</sup>, A. Zanginyan<sup>1</sup>, M. Harutunyan<sup>2</sup>, L. Ghazaryan<sup>2</sup>, A. Nerkararyan<sup>2</sup>, Institute of Molecular Biology NAS RA, Hasratyan st. 7, Yerevan, 375014 Armenia

The study of *Trichinellosis* in Armenia was launched in 1980 and, in effect, covers the whole of the Republic. *Trichinella* larvae have been detected in animals by digestion of muscle tissues. Serodiagnosis in Armenian people was made by enzyme immunoassay (EIA). Overall, 3904 mammals belonging to 24 species have been examined. Of these, 18 species comprising 1821 individuals were identified as wild and 6 species totaling 2083 individuals - as domestic and synantropic. The following species were referred to as *Trichinella* hosts: *Sorex araneus*; *Apodemus sylvaticus*; *Microtus arvalis*; *M. majori*; *Hystrix leucara*; *Canis lupus*; *C. aureus*; *Vulpes vulpes*; *Martes foina*; *Meles meles*; *Felis lynx*; *Sur scrofa*; *Ursus arctos*; *Mus musculus*; *Rattus norvegicus*; *F. domestica*; *C. familiares*; *Alopex lagopus*; *Sus scrofa domestica*. Negative results were obtained in *Dryomys nitedula*, *Colomys mystax*, *Chianomys nivalis*, *M. socialis*, *Myocastor coypus*. The detected helminth species were identified as *Trichinella spiralis* Owen, 1835. The data analysed revealed 7 foci of infection that are basically located in the areas of the republic's montane forest. The natural foci of infection are supported by predators, insectivorous and pair-toed animals, while the synantropic ones are contributed by domestic carnivores, rodents and pair-toed animals. The agent linking the natural and synantropic foci of *Trichinellosis* are domestic pigs kept in the open which is typical of the area's everyday life. The immunologic diagnosis involved virtually 1000 healthy individuals from Yerevan and 8 rural habitats. The percentage of EIA positive results varied between 0 and  $3.92 \pm 1.2$ , averaging  $2.76 \pm 0.72$ . The study suggests that the potential epidemiological susceptibility of people to *Trichinellosis* is most evident in the areas of the country's montane forest. The EIA values for Yerevan approximate those of mountain forest. This fact leads us to believe that the conditions in Yerevan have optimised the susceptibility of the local population to *Trichinellosis*. The results of serodiagnosis among Armenian population for *Trichinellosis* allowed to distinguish areas of high risk (Yerevan, montane forest) and areas of lower risk (mountains-steppe and desert-semidesert habitat).

103. **Risk for *Trichinella* infection in Romanian horses.** C-M Cretu<sup>1</sup>, I. Dida<sup>2</sup>, K. Nockler<sup>3</sup>, E. Pozio<sup>4</sup>, C. Kapel<sup>5</sup> and P. Boireau<sup>6</sup>, “C. Davila” University of Medicine and Pharmacy, Romania, Bucharest<sup>1</sup>, Faculty of Veterinary Medicine, Romania, Bucharest<sup>2</sup>, Federal Institute for Risk Assessment, Germany, Berlin<sup>3</sup>, Istituto Superiore di Sanità, Italy, Rome<sup>4</sup>, Danish Center for Experimental Parasitology, Denmark, Copenhagen<sup>5</sup>, INRA AFSSA ENVA UPVM, France, Maisons Alfort<sup>6</sup>

Despite the control and prophylactic measures, Romanian territory is still endemic for *Trichinella* infection both in humans and animals (domestic and sylvatic). *T. spiralis* and *T. britovi* are the identified species. Romania is also one of the most important horsemeat exporters on EU markets. The risk evaluation of horses to acquire *Trichinella* infection was evaluated by means of serology and morphological examination methods - artificial digestion or trichinelloscopy. During 2002-2003 a number of 3,011 horse sera, coming from different parts of the territory, well known for high incidence of trichinellosis in pigs, have been examined using ELISA E/S antigens. Western blot was the confirmation test for the 17 doubtful or positive samples, one of them being confirmed. After slaughtering and examination of its whole carcass, using trichinelloscopy or artificial digestion, no *Trichinella* larvae have been found. A number of 19,720 horses have been slaughtered in 2 special slaughterhouses (Alexandria and Timisoara), to be exported in EU countries. Trichinelloscopy and artificial digestion have been performed in all cases, but no positive horse was found. We conclude that there is a minimum risk for horses to acquire *Trichinella* infection, even if they are coming from an endemic territory, if natural fodder is recommended and used. Serological screening of horses for *Trichinella* infection proved to have a limited value, probable due to the short persistence of specific antibodies. Work funded by the EU project “TRICHIPORSE” (contract QLK1-CT-2001-01156).

104. **Checking the accuracy of trichinelloscopy in naturally infected pigs with low muscle larvae burden.** R. Beck<sup>1</sup>, Ž. Mihaljević<sup>2</sup>, A. Marinculić<sup>1</sup>, Department of Parasitology<sup>1</sup>, Veterinary Faculty, University of Zagreb, Heinzelova 55, 10 000 Zagreb, Croatia; Veterinary Institute<sup>2</sup>, Savska 143, 10 000 Zagreb, Croatia

Control of *Trichinella* infection in pigs has traditionally been accomplished by inspection of individual carcasses in Croatia. According to the high prevalence, all pigs slaughtered for consumption even for private purposes have to be routinely examined in order to prevent any infection in humans. In this study we tested the accuracy of trichinoscopy in samples obtained from positive pigs slaughtered for private purposes and with lower muscle larvae burden. Among 1769 muscle samples 290 were found infected with 3 or less larvae/g by a routine peptic digestion. The overall accuracy of the trichinelloscopy was 59.48% with the Kappa value of 0.26%. Sensitivity of the test was 43.4% and specificity 88%. Artificial digestion in parallel with the trichinelloscopy revealed a considerable number of previously false negative animals carrying burdens sufficient to cause clinical trichinellosis in humans. This finding confirms previous conclusions that trichinelloscopy is not a method of choice and it is essential to implement another parallel test for post-slaughter control under all conditions.

105. **Detection of *Trichinella spiralis* in Zacatecas, Mexico.** Jesús Muñoz- Escobedo<sup>1</sup>, Gabriela Reveles-Hernández<sup>2</sup>, Sergio Saldivar- Elias<sup>2</sup>, Alejandra Moreno-García<sup>2</sup>. <sup>1</sup>Unidad Académica de Odontología. Universidad Autónoma de Zacatecas; <sup>2</sup>Unidad Académica de Biología Experimental. Universidad Autónoma de Zacatecas. México. Apartado Postal 12. Guadalupe Zacatecas. México. CP. 98600

Trichinellosis is and endemic zoonosis in Zacatecas state that is transmitted to human by eating pig meat contaminated with *Trichinella spiralis*. This endemic zoonosis has been reported since 1976. Objective: To detect *T. spiralis* in the hosts that allow its persistence. Methods and Materials: We studied 200 live pigs of (6) six communities from Zacatecas, 100 sacrificed pigs in Zacatecas and Jerez, 100 rats from the municipal trash, and in tongues from 100 dogs sacrificed in the canine center of Zacatecas. We detected *Trichinella spiralis* by the direct techniques of tissue compression and artificial digestion, and by the indirect techniques Dot-ELISA and Western blot. Results: In the sacrificed pigs we found by direct techniques one positive, and by indirect techniques six positive sera detecting a triplet of 42, 45 and 48 kDa. In live farm pigs, we found (by indirect techniques) ten positives detecting a triplet of 42, 45 and 48 kDa. In rats we found by direct and indirect techniques, three positives detecting the mentioned triplet. In dog tongues by direct technique we found three positives. From the positive tissues, we reproduced the parasite's life cycle in mice. Conclusion: *Trichinellosis* is present in hosts that allow its permanence as a zoonosis in Zacatecas, México.

106. **Evaluation of ELISA for detection of trichinella antibodies in muscle juice samples of naturally infected pigs.** R. Beck<sup>1</sup>, A. Gašpar<sup>3</sup>, Ž. Mihaljević<sup>2</sup>, A. Marinculic<sup>1</sup>, D. Stojčević<sup>1</sup>, M. Brstilo<sup>3</sup> Department of Parasitology<sup>1</sup>, Veterinary Faculty, University of Zagreb, Heinzelova 55, 10 000 Zagreb, Croatia; Veterinary Institute<sup>2</sup>, Savska 143, 10 000 Zagreb, Croatia; Ministry of Agriculture and Forestry Republic of Croatia<sup>3</sup>, Ulica Grada Vukovara 78, 10 000 Zagreb, Croatia

The performance characteristics of an ELISA test for trichinellosis in pigs applied to muscle juice was assessed using 314 samples collected from pigs located in endemic area of Croatia. The peptic digestion assay was regarded as the reference method. The diagnostic accuracy of the two compared dilutions (1:10 and 1:100) was found high because the index AUCs was 0.922 and 0.920, respectively. In this study the two-graph receiver operating characteristic (TG-ROC analysis was used as a tool for selecting cut-off points. Sensitivity, specificity, likelihood ratios, efficiency and Youden's index were used as indices of test accuracy. The cut-off values that minimize overall misclassification cost under assumption of 3% prevalence were calculated. Our results indicate that the ELISA applied to muscle juice is a highly accurate test and can be adapted to process a large number of samples.

**107. Meat juice of infected pigs as a source for specific *T.spiralis* antibody detection.** Lj. Sofronic Milosavljevic<sup>1</sup>, M.Petrovic<sup>1</sup>, M. Djordjevic<sup>2</sup>, M.Savic<sup>2</sup>, K. Cuperlovic<sup>2</sup>, I.V. Patrascu<sup>3</sup>. Institute for the Application of Nuclear Energy - INEP<sup>1</sup>, Institute of Meat Hygiene and Technology IMHT<sup>2</sup>, 11080 Belgrade, Serbia and Montenegro, A.R.T.E. Int. SRL<sup>3</sup>, 77713 Bucharest, Ro

Infection with *T.spiralis* provokes a strong and consistent antibody response that could be detected in sera or body fluids of swine. Since lateral flow card test (TS Card Pork, IVD, USA and ARTE.SRL. Ro) proved to be almost as specific and sensitive as ELISA (but more simple and easy to perform) for anti -*Trichinella* IgG detection in swine sera; the aim of this study was to evaluate its applicability for antibody detection in meat juice of infected pigs at slaughter. Animals originating from small individual farms located in *Trichinella* endemic region of Sid, Serbia, were slaughtered and meat juice samples were collected individually from 5 g of frozen diaphragm meat sample, by originally designed meat pressure system. The double testing of meat juice samples was performed by TS Card Pork and ELISA. For final judgment on tests results parasitological findings were used. From 52 carcasses: 35 were detected as *Trichinella* infected by peptic digestion method, from which 27 by 1 g of meat sample and 8 by 25 or 50 g of meat sample. In conditions of positive findings in 1g of the meat sample digestion the confidence level between digestion and TS Card pork reached 100%, while with ELISA it was 92.5%. Testing samples from pigs with very low level *Trichinella* infection (less then 1 LPG) revealed that TS Card level of test sensitivity could be established at 0.12 LPG of muscle. Therefore “TS Card Pork” test could be used as an intermediate method for identifying individually infected animals in aim to reduce expense and time due to long procedure of digestion re-examination.

**108. Study concerning pathomorphological aspects in larvae and cysts of *Trichinella spiralis* in swine meat.** Ioan Cironeanu, PhD, Bucharest, Romania, Tel. 0040.2329350

The study, which has been made in slaughterhouses and specialised laboratories for many years, has shown a large variety of features in *Trichinella spiralis*. An inflammatory infiltrate of variable cysts of intensity generates essential modifications in the structure, the form and the dimensions of *T. spiralis* cysts. Frequently, such modifications raise serious problems in the diagnosis at the trichinelloscopic examination. Following the development of larvae in muscles, there are a large number of morphological and pathomorphological aspects, as a consequence of the local inflammatory reaction and of the evolution from the stretching, coiling and capsule formation stages to the calcification process. For avoiding diagnosis errors, with serious economic and public health effects, the controller must have, besides highly performing instruments, a sound theoretical and practical expertise regarding the whole range of the aspects of *Trichinella spiralis* in the muscle tissue.

**109. An accreditation program for reliable *Trichinella* testing of pork and horsemeat by private industry in Canada.** W. B. Scandrett, L. B. Forbes, and A. A. Gajadhar, Centre for Animal Parasitology, Saskatoon Laboratory, Canadian Food Inspection Agency, Saskatoon, Saskatchewan, Canada S7N 4N2

The Centre for Animal Parasitology, Canadian Food Inspection Agency, maintains a quality assured system for *Trichinella* testing of pork and horsemeat based on a fully validated and published digestion assay. The system is monitored via proficiency sample use and is accredited by the Standards Council of Canada (SCC) in accordance with ISO 17025 guidelines. An accreditation program based on this system has been developed and implemented for the detection of *Trichinella* in pork and horsemeat by private industry laboratories to fulfil export requirements to Eastern Europe and the European Union, respectively. Accredited laboratories must meet specific requirements demonstrating a satisfactory quality test system, including provision of a QA manual, standard operating procedures (SOPs) and associated documentation, adequate facilities and equipment, and analyst proficiency in performance of the *Trichinella* digestion assay. Maintenance of accredited status is contingent on continued compliance with these requirements. An overview of this external *Trichinella*-testing accreditation will be presented, including proficiency evaluation of analyst competence. Ongoing efforts to further optimize and standardize the program in support of quality assurance, biosafety, and test method will also be discussed.

**110. A control program to reduce the risk of infection with *Trichinella spiralis* in New Zealand pigs.** E.K.B.Richardson<sup>1</sup>, D.E.Lawton<sup>1</sup>, M.A.Potter<sup>2</sup>, Epicentre, Institute of Veterinary, Animal and Biomedical Sciences<sup>1</sup>, Ecology Department<sup>2</sup>, Massey University, Palmerston North, New Zealand

Trichinellosis is an extremely rare zoonotic disease in New Zealand. Only four human cases have been detected in the past. These cases were all traced to pigs reared in 'back-yard' production systems, and routine sampling of commercially raised pigs over the past 25 years has not revealed evidence of infection. Despite its rarity, *Trichinella spiralis* has become an issue for the development of an export market for chilled pig products. Therefore this study investigated the prevalence of *T. spiralis* by the pepsin digest method in purposively selected populations of rats from commercial piggeries, Department of Conservation reserve land, and waste disposal landfills throughout New Zealand. No positive samples have been found in this test series. Since rodents are considered an important reservoir of *T. spiralis*, the efficacy of a comprehensive rodent-baiting program was monitored on three commercial piggeries over 16 months. Rodent activity was measured using tracking tunnels distributed evenly across each farm. These tunnels use a non-toxic lure to attract small animals into the tunnel, recording their footprints. Farm hygiene and compliance with the baiting programme strongly affected the success of rodent control. The study gave a picture of rodent activity and its spatial distribution between buildings and production groups in the course of intensive baiting. These studies were used to formulate a New Zealand industry quality assurance program to ensure the absence of *T. spiralis* infection in commercial piggeries.



111. **Detection of *Trichinella* infection in field pigs in Argentina using the ELISA test.** M. Ribicich, H. R. Gamble, A. Rosa, I. Sommerfelt, J. Bolpe, H. Torno, M. Verdier and A. Franco. Parasitology and Parasitic Diseases. Facultad de Ciencias Veterinarias. Universidad de Buenos Aires, Chorroarín 280. (C1427CWO) Ciudad de Buenos Aires, Argentina

Serological methods, in particular the ELISA, provide opportunities for rapid detection of *Trichinella* infection in pigs and humans. For pigs, the ELISA has the advantage that it may be used prior to slaughter to detect infection. Argentina is an endemic area for *Trichinella* infection in pigs and trichinellosis in humans. Santa Fe, Buenos Aires and Córdoba provinces are among those areas most affected. Serum samples were collected from 1159 pigs from farms in these three provinces. Pigs were selected from farms that included total confinement management, pigs raised outdoors, and animals raised under poor hygienic and sanitary conditions. All samples were tested to detect anti-*T. spiralis* antibodies in an ELISA using *T. spiralis* ES antigens and a synthetic glycan antigen. In addition, 100g of diaphragm tissue from each pig was processed by artificial digestion to correlate the presence of *T. spiralis* larvae with ELISA results. From the total number of pigs, 18 (1.55 %) were found to have *T. spiralis* larvae by artificial digestion. Worm burdens in infected pigs ranged from 8.4 to 105.6 larvae per gram. Agreement between serological methods (using ES and glycan antigens) and digestion had a Kappa index of 1.0 and 0.97, respectively. Overall, serological prevalence was 0%, in pigs raised in confinement or in the field, under controlled sanitary conditions, while it was of 9.27% for pigs raised under poor hygienic and sanitary conditions. The ELISA method is recommended for the herd surveillance programs; it is useful for detecting ongoing transmission of *Trichinella* at the farm level in Argentina, and it can be a useful tool for assuring the safety of meat to the consumer.

112. **On fundamental problems of trichinellosis in man and animals in Romania.** Gh. Olteanu, I. Cironeanu, Laboratory of Parasitology of A.P.R., Bucharest, Prevederii, 20, Bl. G3,ap.25, cod 032303, Romania

The first case of Trichinellosis in Romania was diagnosed in man in 1868. During last months of 1869 disease had been experimentally transmitted from man to pig, dog and cat. In 1962 *T. pseudospiralis* was detected in *Corvus frugilegus*. Trichinellosis had been registered 3 periods. 1) 1868-1983: had been similar with situation in European Countries; 2) 1983-1993: numbers of cases registered a strong increase: a) in man-from 217 cases in 1982 to 3649 in 1993 (17 fold increase); b) in pigs 10540 cases (0.22%) in 1993; 3) After 1994, number of cases with *Trichinella* in man and pigs began to decrease concomitant with a decrease of number of animals. The cause of strong increase (1983-1993) had been *Trichinellosis* in pigs in the big industrial farms. During 1984-1986, more 95% cases of *Trichinellosis* in pigs in Romania had been in big industrial farms. From farms with *Trichinella* many thousand pigs dispersed to many small farms. During 1991-1995, many industrial farms with *Trichinellosis* were dissolved. It had been demonstrated that *Trichinellosis* in pigs may be eradicated when this disease was concentrated in big industrial farms. For this there is an original method of Complex Chemoprophylaxis of Trichinellosis. By application of this method *Trichinellosis* had been eradicated in 11000 pigs (21.6%) from Crivesti-Tutova farm during 7 months (1973). In the new conditions of the last decade, the eradication of Trichinellosis is necessary and possible, but is more difficult.

113. **Serological evidence of trichinellosis in local pigs of Nepal.** D.D. Joshi<sup>1</sup>, L.N. Moller<sup>2</sup>, M. Maharjan<sup>1</sup>, C.M.O. Kapel<sup>2</sup>, <sup>1</sup>National Zoonoses and Food Hygiene Research Centre Tahachal, Kathmandu, Nepal, <sup>2</sup>Danish Centre for Experimental Parasitology, Denmark.

In Nepal, animal husbandry is a major source of income. Pig husbandry is present both in rural, peri-urban, and urban communities and free ranging “back yard” pigs and the practice of offal feeding is very common. Trichinellosis have never been reported from this region, but the pig management practice should potentially allow for the transmission of the disease. A total of 425 serum samples collected from local pigs were initially screened by ELISA (ES and Tyvelose antigens) after which positive samples were examined by Western Blot. This procedure identified a few samples which had clear specific bands for *Trichinella*. Although, 52 meat samples tested by HCL-pepsin digestion were found to be negative, the highly specific serological analysis indicate that trichinellosis is present in Nepal. An eventual prevention program should aim to prevent the access of pigs to open garbage dumps which exist both in towns and on farms.

114. **Intensity of *Trichinella* sp. infection in the pig.** Gh. Cristea, Debora Cristea<sup>2</sup>, Eugenia Cristea, The Veterinary District Vulcan, N.Titulescu Street, No.42,336200-Vulcan, Romania; <sup>2</sup>The Medicine and Pharmacy University Carol Davila, Bucharest; <sup>3</sup>The Individual Medical Cabinet, No.227, N.Titulescu Street

A number of 426 *Trichinella* larvae infested pig hulls were examined and were identified cysts and/or free *Trichinella* larvae in the external muscles of the tongue in 419 animals (98.35%), in the midriff muscles in 386 pigs (90.6%) in the following muscle groups: the radial midriff muscles- 339 heads(79.57%), the intercostals muscles- 334 heads (78.40%), the forearm’s muscles- 295 heads (69.24%), the dorsal muscles- 191 heads, the flank muscles- 169 heads (39.67%), the ear’s muscles- 98 heads (23%), the scut’s muscles- 72 heads (16.90%). We effected a compressed blade with 28 microscope fields from each region of the body and we counted all the cysts and the free *Trichinella* sp. larvae. Processing statistical data, we noticed the maximal intensivity in the external muscles of the tongue- 45%, following in order: the midriff muscles- 13.2%, the radial midriff muscles- 11%, the intercostals muscles- 9.2%, the forearm’s muscles- 6.9%, the cervical muscles- 6%, the dorsal muscles – 3.3%, the flank muscles- 2.8%, the ear’s muscles- 1.35% and the scut’s muscles- 1.25%. in the infested naturally boar, the maximal intensivity was found in the external muscles of the tongue and in the midriff muscles; in the brown bear and in the rat, the most infested muscles were the tongue’s muscles and the internal masseterian muscles; in the mice the maximal intensivity was found in the flank muscles (the inferior abdominal muscles) and in the masseterian muscles, in fox the most infested muscles were. the eye’s muscles and the forearm’s muscles; in dogs the maximal intensivity was in the deep cervical muscles and in the forearm’s muscles.

115. **The French National Reference Center on *Trichinella*** . J. Dupouy-Camet and T. Ancelle. Parasitology Department, Hôpital Cochin, Université R. Descartes, 27 Fbg St Jacques, 75014 Paris, France

The national reference center monitors human trichinellosis as recommended by the European Union and french ministry of health authorities. Between 1975 and 2003, 24 outbreaks including at least 2429 cases emerged in France. 95% of cases were observed during eight outbreaks caused by horse meat consumption. The objectives of the national reference center are: 1. to detect asap outbreaks and to alert the proper authorities 2. to count the annual cases with the help of a network of 37 medical parasitology laboratories of university hospitals and of three major private laboratories performing serological assays all over France. 3. to help to the parasitologic and serologic diagnosis (western-blot of sera to eliminate cross reactions and molecular typing of isolates) 4. to give therapeutic advices based on "Opinion one the diagnosis and treatment of human trichinellosis" (Expert Pharmacother opinion. 2002, 3,1117-30). A web site provides informations on the disease and a form to declare cases (<http://monsie.wanadoo.fr/cnrdestrichinella/>). The national reference center works in close cooperation with the veterinary reference laboratory of the AFSSA (UMR BIPAR, Pascal Boireau) and with the International Reference center (ISS, Rome). After the large outbreaks of 1998, only imported sporadic cases (Africa, eastern Europe) or small family outbreaks (wild boar hunters) were reported. Two cases were identified in 1999, 0 in 2000, 2 in 2001, 5 (one outbreak of 4 cases) in 2002, and one outbreak of 6 cases in 2003. The training of the technicians in charge of veterinary control, implemented by AFSSA, avoided 2 outbreaks since two infected horse could be intercepted in 1999 and 2001.

116. **Necessity for the application of quality assurance (QAS) and proficiency samples programs in meat inspection for trichinellosis.** M. Djordjevic, K. Cuperlovic, M. Savic, S. Pavlovic, Institute for Meat Hygiene and Technology, 11000 Belgrade, Kacanskog 13, Serbia and Montenegro

Introduction of the ICT recommendations for the methods for *Trichinella* control in meat and particularly introduction of quality assurance and proficiency samples for certified analysts in Serbia, as well in other countries, will be of great importance. The introduction the mentioned systems will prevent the disease in humans after consummation of inspected meat. In spite of existing regulations and inspection of 0.5 g of diaphragm meat by compression method or 1g by artificial digestion method, in 2001/2002 in Serbia 280 people showed clinical symptoms after consummation of the inspected meat in 3 municipalities: Kumane, Surcin and Bogatic. This was the consequence of improvisation and non-adequate application of the methods and regulations by some analysts and insufficient education of some veterinary inspectors in their line of work. In the past decade such events also took place in some regions of former Yugoslavia.

117. **Successful eradication of swine trichinellosis in highly endemic village in Croatia.** Balić Davor<sup>1</sup>, Albert Marinculic<sup>2</sup>, Relja Beck<sup>2</sup>, Croatian Veterinary Institute<sup>1</sup>, Josipa Kozarca 24, 32100 Vinkovci, Croatia; Department of Parasitology<sup>2</sup>, Veterinary Faculty, University of Zagreb, Heinzlova 55, 10 000 Zagreb, Croatia

Uncontrolled conditions of slaughter and disposal of infected carcasses provoked an increase of the prevalence of trichinellosis in swine in previously known endemic village in the region of Eastern Slavonia. The aim of this study was to describe the conditions of the decline of the disease due to severe obligatory control measures during the period from January 1999 to January 2004. Because of the possibility of rapid spread as was seen in other parts of the country, a disease control plan was implemented in 2000 which included routine trichineloscopy of all slaughtered swine and permanent rodent control. Additionally prompt disposal (in the period of 12 hours) of infected carcasses was instituted to reduce the access of swine, rodents or other animals to potential sources of infection. An important component of the program involved monitoring the prevalence of *Trichinella* infection in order to determine the effect of control measures. A trichinellosis rate of 3,56% (69 infected swine from 29 small private farms among 2442 samples controlled by routine trichineloscopy) existed in swine prior to implementation of control measures. According to the previous data on the foci of trichinellosis in swine the Ministry of Agriculture and Forestry promulgated a decree of obligatory slaughtering under controlled conditions (in well equipped local slaughterhouse) of all animals from farms previously detected as foci. 492 swine were slaughtered. Among them, 18.69% were found infected. It has to be stressed that as a result of severe control measures, the prevalence of *Trichinella* decreased by 3.56% in 1999 to 0% in 2004.

118. **Comparison of two antigens for demonstration of *Trichinella* spp. antibodies in blood and muscle fluid of foxes, pigs and wild boars.** L.N. Møller<sup>1,2</sup>, E. Petersen<sup>2</sup>, H.R. Gamble<sup>3</sup> and C.M.O. Kapel<sup>2</sup>. <sup>1</sup>Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark. <sup>2</sup>Department of Bacteriology, Mycology and Parasitology, Statens Serum Institut, Copenhagen, Denmark, <sup>3</sup>US Department of Agriculture, Beltsville, Maryland, USA

Serological detection of *Trichinella* in meat products has yet not proved to be an efficient way to certify pork, but it has a strong potential for surveillance in production animals and wildlife. For such surveillance, blood serum is usually used, although muscle fluid can be a good alternative due to a more simple sampling procedure especially considering wildlife sampling. The only disadvantage is the fact that antibodies are present at a lower level compared to blood sera. In the present study, we evaluated an indirect ELISA technique employing both sera and muscle fluids from experimentally infected foxes, pigs, wild boars. The three host species used in this study were infected in groups with seven well defined *Trichinella* genotypes, and ELISA was made on both sera and muscle fluids using an E/S antigen and a synthetic glycan antigen tyvelose. The detection of IgG antibodies in both serum samples and the muscle fluid matrix showed comparable results from infected pigs, wild boars and foxes, although some differences were detected in the sensitivity of the two antigens.

119. **Comparison of two iELISA procedures for early detection of specific *Trichinella* antibodies.** W. Cabaj<sup>1</sup>, B. Moskwa<sup>1</sup>, J. Bien<sup>1</sup>, K. Pastusiak<sup>1</sup>, J. Pourquier<sup>2</sup>, K. Nöeckler<sup>3</sup>, F. J. Serrano<sup>4</sup>, E. Pozio<sup>5</sup>, <sup>1</sup>Witold Stefanski Institute of Parasitology of the PAS, Twarda 51/55, 00-818 Warsaw, Poland. <sup>2</sup>Institut Pourquier, 326 rue de la Galera, 34000 Montpellier, France. <sup>3</sup>Bundesinstitut für Risikobewertung, Diederdorfer Weg1, D-12277 Berlin, Germany. <sup>4</sup>Facultad de Veterinaria Universidad de Extremadura Avda. de la Universidad, s/n, 10071-Caceres, Spain. <sup>5</sup>Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Roma, Italy

The official recommended methods of trichinellosis detection at the slaughterhouse make it one of the most costly of all zoonoses. The aim of this work was to compare the usefulness of two iELISA procedures for early serological diagnosis of specific *Trichinella* antibodies before slaughter. Conventional, Iberian pigs were inoculated with 200, 1000 and 20 000 muscle larvae of *T. spiralis*. Serum samples were obtained at -1, 5, 10, 15, 20, 25, 30, 40, 50 and 60 dpi and screened for specific IgG antibodies to excretory/secretory L1 *T. spiralis* antigen (ES L1 *T. spiralis* Ag). The results have shown that for sera from pigs tested with ES L1 *T. spiralis* Ag prepared in two different laboratories, the earliest positive response appeared on 25 dpi but only in groups of pigs infected with the highest dose of larvae. Comparable results were obtained using both, Standard and General procedures. Additionally, very similar pattern of immune response was observed when different procedures and different antigens were used. The highest dilution of examined sera and components used in General Procedure resulted in the lower level of OD.

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120. **Specific diagnostic antigens in ES products from *T. spiralis* muscle larvae.** Z.Q.Wang<sup>1</sup>, J.Cui<sup>1</sup>, D.Zhang<sup>1</sup>, H.Y. Wei<sup>1</sup>, B.L.Xu<sup>2</sup>. Department of Parasitology, Medical College, Zhengzhou University, Zhengzhou 450052<sup>1</sup>, Health and Anti-epidemic Center of Henan Province<sup>2</sup>, Zhengzhou450003<sup>2</sup>, China

To find out specific diagnostic antigens in excretory-secretory (ES) products from muscle larvae of *Trichinella spiralis*, ES antigens of *T. spiralis* larvae cultured in vitro at 18 h and 30 h were analyzed by SDS-PAGE and Western blot. The Results showed that protein components of ES antigens were similar after different culture times. SDS-PAGE revealed that the molecular weight (MW) of major bands of 2 ES antigens were 112,110,108,97,53,49,45,42,35,23,16 kDa. Western blot showed that all of the protein bands with 102,97,95,53kDa in 18 h ES antigens and the protein bands with 53,49,45,43 kDa in 30 h ES antigens were cross-reacted with sera from the patients with paragonimiasis, clonorchiasis, schistosomiasis, and cysticercosis, respectively. The protein component with 23kDa in ES antigens were only reacted with sera from the rats and mice infected with *T. spiralis* and the patients with trichinellosis. The results suggested that the major protein components 53,49,45,43kDa of ES antigens used for serodiagnosis of trichinellosis in European and northern American countries might not be adapted in China, because the named parasitic diseases are rare in developed countries, but common in China. The 23 kDa protein in *T. spiralis* ES antigens could be applied to the serodiagnosis and seroepidemiological survey of trichinellosis in China.

121. **Common antigens among *T. spiralis*, *P. westermani* and *C. sinensis*.** Z.Q.Wang, J.Cui, D.Zhang. Department of Parasitology, Medical College, Zhengzhou University, Zhengzhou,450052, P.R.China

To identify the common antigens among *Trichinella spiralis*, *Paragonimus westermani* and *Clonorchis sinensis*, and to avoid the cross-reaction of serodiagnosis of the three parasitic diseases, the soluble antigens of *T. spiralis* muscle larvae, *C. sinensis* and *P. westermani* adult worms were analyzed by SDS-PAGE and Western blot. The results showed that there are the same protein bands in the soluble antigens of *T. spiralis* larvae, *P. westermani* and *C. sinensis* adult worm, their MW are 108,65,53,43,42,31,25,16 kDa. Immunoblotting showed that all of the protein bands with 65,58,53 kDa in both *T. spiralis* and *P. westermani* soluble antigens reacted with sera from rats, mice and patients with trichinellosis, and sera from rats and patients with paragonimiasis; the protein bands with 108 kDa in both *T. spiralis* and *C. sinensis* soluble antigens reacted with sera from rats, mice and patients with trichinellosis, and sera from patients with clonorchiasis. The protein component with 53 kDa in the above-mentioned three soluble antigens reacted with all sera from animals and patients infected with parasites in this experiment. We conclude that the 65, 58 and 53kDa proteins were the common antigens between *T. spiralis* and *P. westermani*, the 108kDa protein was the common antigen between *T. spiralis* and *C. sinensis*, and the 53 kDa protein was the common antigen among *T. spiralis*, *P. westermani* and *C. sinensis*.

122. **Two dimensional electrophoresis and mass spectrometry (MALDI TOF) for the identification of species-specific *Trichinella* antigens.** M. A. Dea-Ayuela and F. Bolás-Fernández. Departamento de Parasitología. Facultad de Farmacia, Universidad Complutense. Ciudad Universitaria. 28040-Madrid. España

A proteomic approach was applied for fine antigenic characterization of the closely related *Trichinella* genotypes T3 (*T. britovi*) and T8. Soluble proteins of muscle larvae L1 (SPL) from both isolates were extracted by sonication, and subsequently analyzed by two dimensional polyacrylamide gel electrophoresis (2D-PAGE) using an immobilizer linear pH 3-10 gradient for isoelectric focusing. Over 400 protein spots were reproducibly separated and the comparative analysis of 2-D gels revealed similar profiles of protein expression between both species. These separated proteins were proved in western-blot with hyperimmune sera raised in BALB/c mice following immunization with SPL from each of the 2 species. About 20 and 15 cross-reactive proteins were revealed by the IgG1 and IgG3 isotype subclasses respectively, that were situated around pI 4-7 and 80-40 kDa MW. In *T. britovi*, a group of 6 acidic proteins, in the range of 30-40 kDa MW, were revealed by the IgG1 raised only in the homologous sera. MALDI-TOF and MALDI-TOF/TOF MS mass spectrometry analysis of these antigens allowed to identify an enolase, the protein P49 and an actin among the cross-reactive proteins and 2 hypothetical actins among the non cross-reactive proteins.

123. **The status of trichinellosis in Uzbekistan.** M. Aminjonov, Uzbek Veterinary Scientific Research Institute, Samarkand, Republic of Uzbekistan

Trichinellosis can be a cause of significant economic problems in countries where pig production is a major industry. Human disease results in serious illness or death. As a result, trichinellosis is both a veterinary and a medical problem. There was a great deal of attention paid to detecting *Trichinella* infection in pigs in republics of the former Soviet Union, including Uzbekistan. The number of pigs produced in Uzbekistan decreased after the dissolution of the Soviet Union. Today, fewer pigs are produced and many former pig enterprises are no longer in operation. No human cases of trichinellosis were reported during the period when Uzbekistan was part of the Soviet Union. However, testing of pork continues at private sector markets. Testing is conducted by the trichinoscope method, using samples obtained from the tongue, masseter or intracostal (rib) muscles. The detection of *Trichinella* in rib meat was validated using biological samples from mice and rats, which were infected with *Trichinella*. Results demonstrated that compression testing, using the trichinoscope, can be used with rib meat samples, as a reliable method for detecting *Trichinella* infection in pigs. No cases of trichinellosis in pigs have been reported in Uzbekistan when it was part of the Soviet Union nor after it gained independence. Generally, the people of Uzbekistan are Muslims, who are forbidden to eat pork; this factor contributes to the absence of trichinellosis among the Uzbeki people.

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# Abstracts - Author Index

Akimov, I.A.	67	Cironeanu, I.	108, 112	Gruden-Movsesijan, A.	53, 89
Alcaraz, A.	54	Connolly, B.	21	Guiliano, D.B.	20
Aminjonov, M.	123	Coss, C.	64	Gurish, M.F.	40
Ancelle, T.	115	Costantino, S.N.	26, 72, 73	Han, H.M.	43, 45
Andersen, S.	62	Cote, M.	46, 47	Hang, H.M.	25
Andreyanov, O.N.	69	Cretu, C-M.	103	Harutunyan, M.	102
Angeles-Angiano, E.	39	Cristea, D.	65, 96, 97, 114	Hernández-Bello, R.	22
Appleton, J.A.	35, 50, 54	Cristea, E.	65, 96, 97, 114	Hernández-Campos, A.	71
Arizmendi, N.	41, 42	Cristea, Gh.	65, 97, 114	Hernández-Luis, F.	71
Asatrian, A.	102	Cruz, I.V.	71	Hernandez-Sanchez, J.	48, 49
Ascarateil, S.	46	Cui, J.	25, 43, 44, 45, 120, 121	Hill, D.	10, 64
Ashrafi, K.	30	Cuperlovic, K.	89, 93, 107, 116	Hoberg, E.	1
Austen, K.F.	40	Damjanovic, S.	93	Holowka, D.	50
Bahuon, C.	19	Darabus, G.	74, 91	Hotez, P.	11
Barbosa-Cisneros, O.Y.	36, 37	Davila, C.	103	Hu, M.	8
Barrionuevo-Samaniego, M.	101	de Borchgrave, J.	101	Huber, D.	70
Basurto Frausto, Ma.G.	36, 37	De Bruyne, A.	9	Hui, L.	88
Beck, A.	38, 70	de Pooter, A.	46	Hurnikova, Z.	5, 7
Beck, R.	70, 99, 104, 106, 117	de Vries, A.	58	Iacobiciu, I.	74, 91
Befus, D.	42	Dea-Ayuela, M.A.	122	Ilic, N.	89
Beijing, D.P.	35	Derdoy, M.	73	Järvis, T.	63
Benić, M.	99	Deville, S.	46	Jasmer, D.P.	33
Benítez-Ortiz, W.	101	Dida, I.	4, 103	Jimenez-Cardoso, E.	39, 95
Bermúdez-Cruz, R.M.	22	Didyk, J.M.	67	Jokela, S.	59
Bessonov, A.S.	69, 100	Djordjevic, M.	89, 93, 107, 116	Joshi, D.D.	113
Bianli, X.	88	Dorny, P.	101	Kapel, C.M.O.	5, 6, 7, 13, 14, 15, 17, 23, 24, 32, 39, 61, 62, 63, 94, 101, 103, 113, 118
Bien, J.	68, 119	Dubinsky, P.	4	Keidans, P.	63
Björkroth, J.	3	Dupouy-Camet, J.	9, 80, 115	Kisselgof, A.B.	40
Blaga, R.	4	Eddi, C.	63	Koch, A.	61, 62
Bliss, S.K.	54	El-Osta, Y.G.A.	8	Kociecka, W.	81
Boireau, P.	7, 9, 11, 13, 14, 15, 17, 19, 22, 46, 47, 51, 103	Enache, G.	77, 78, 79	Koort, J.	3
Bolás-Fernández, F.	122	Enciso, J.A.	42	Koreck, A.	74, 91
Bolpe, J.	90, 111	Epitaño, M.R.	49	Kozek, W.J.	52
Bongiorni, F.	83	Esquivel-Aguirre, D.	49	Kuska, J.	70
Boonmars, T.	28	Finnigan, J.	64	Kwak, D.	33
Bosnić, S.	99	Fonseca-Liñan, R.	12, 51	La Rosa, G.	1, 4, 18, 31, 60
Boyd, P.	10	Fonville, M.	2, 58	Lainé-Prade, V.	46
Brandt, J.	101	Forastiero, M.A.	72	Laurberg, P.	62
Briels, I.	2, 58	Forbes, L.	66, 94, 109	Lawton, D.E.	110
Britov, V.A.	84	Franco, A.	29, 90, 111	Le Guerhier, F.	9, 19, 22, 47
Brstilo, M.	106	Friend, D.	40	Le Naour, E.	19
Bruschi, F.	34, 80, 81, 82, 83	Fronzizi, D.	82	Le Rhun, D.	19
Bryce, P.	40	Frongillo, R.F.	82	Li, C.Y.	17
Cabaj, W.	4, 68, 81, 119	Fu, B.Q.	13, 14, 15, 17	Li, D.	11
Caballero García, M.L.	39, 95	Gajadhar, A.A.	66, 94, 109	Li, L.R.	13, 14
Calcagno, M.A.	72	Gamble, H.R.	5, 6, 29, 59, 64, 90, 98, 111, 118	Li, R.L.	43, 45
Calvo, M.I.	73	García-Mayorga, E.	56	Lichtenfels, J,R,	10, 98
Cardillo, N.	29	García-Reyna, P.B.	22, 51	Liu, M.Y.	13, 14, 15
Casini, A.	34	García-Zepeda, E.	41	Locci, M.T.	81
Castagna, B.	81, 82	Gare, D.	21	Lu, Q.	15, 17
Castillo, R.	71	Gašpar, A.	106	Ludovisi, A.	75
Castillo Alvarez, A.M.	12	Gasser, R.B.	8	Lugo- Hernández, S.	41
Casulli, A.	76	Gay-Jiménez, F.R.	95	Lukashkova, I.N.	84
Cattaneo, M.L.	29	Gentile, T.	26, 73	Luna Sánchez, B.	36, 37
Chapa-Ruiz, R.M.	39	Georgiadou, V.	39	Maharjan, M.	113
Chávez- Ruvalcaba, I.	85, 86	Ghazaryan, L.	102	Mahdavi, M.	57
Chávez-Larrea, M.A.	101	Gomez Morales, M.A.	75, 76	Malakauskas, A.	5, 7, 32, 63
Chen, Q.J.	13, 14, 15, 17	González, J.	71	Malczewski, A.	68
Cheng, X.	33	González-Bonilla, C.	12	Maldonado-Tapia, C.	55
Christensson, D.	4	Gounaris, K.	20	Marincu, I.	74, 91
Chute, M.B.	10	Gremigni, V.	34	Marinculic, A.	38, 70, 99, 104, 106, 117

Marquez, A.	29	Robinson, M.W.	21
Martinez-Abrajan, D.M.	49	Rodríguez-Hidalgo, R.	101
Marucci, G.	4, 18, 60, 70	Roman-Díaz, R.	56
Masetti, M.	81	Ron-Román, J.	101
Massoud, J.	30, 57	Rosa, A.	29, 90, 111
Mateo-Gonzales, R.	39	Rosenthal, B.	1
Mazzoni, S.	83	Rossi, L.	34, 60
Measures, L.	94	Ruiz-Olvera, P.	12
Melbye, M.	61, 62	Saldívar-Elías, S.	55, 86, 87, 105
Mendez-Loredo, B.E.	49	Salinas-Tobon, M.R.	48, 51
Meng, X.P.	15	Salvetti, A.	34
Migliorini, P.	83	Sánchez-Rodríguez, S.H.	36, 37
Mihaljevic, Z.	104, 106	Savic, M.	89, 93, 107, 116
Miheli, D.	38		
Mikkonen, T.	3	Saviozzi, M.	34
Miller, H.R.P.	40	Scandrett, W.B.	109
Mira, G.	29	Schmalhausen, I.I.	67
Mobedi, I.	30	Selkirk, M.E.	20
Møller, L.N.	7, 61, 62, 94, 101, 113, 118	Serrano, F.J.	4, 47, 119
Moreno García, A.	36, 37, 55, 56, 85, 86, 87, 105	Serrano Aguilera, F.J.	92
		Sever, K.	38
Moskwa, B.	68, 81, 119	Sofronic Milosavljevic, Lj.	53, 89, 93, 107
Mowlavi, G.	30	Soleymani Mohammadi, S.	30
Muñoz- Escobedo, J.	55, 56, 85, 86, 87, 105	Sommerfelt, I.	111
		Soria, O.	71
Murrell, K.D.	89, 93	Steeves-Gurnsey, T.	66
Naddaf, S.	30	Stojčević, D.	106
Nagano, I.	28	Sukura, A.	3, 59
Näreaho, A.	59	Takahashi, Y.	28
Navarrete-Leon, A.	48	Tao, H.	40
Negrutiu, L.	74, 91	Teunis, P.	58
Nerkararyan, A.	102	Theodoropoulos, G.	39
Nivin, E.A.	84	Thornton, E.	40
Nöeckler, K.	4, 7, 47, 92, 103, 119	Thrasher, S.	50
		Tommasi, S.	83
Nuñez, G.G.	26, 73	Torno, H.	111
Oettgen, H.C.	40	Trejo-Hernández, E.	95
Oivanen, L.	59	Uribe-Gutiérrez, G.	95
Olariu, R.	74, 91	Vallé, I.	46, 47, 51
Olteanu, Gh.	112	van der Giessen, J.W.B.	2, 58
Ortega Pierres, M.G.	12, 22, 41, 42, 51	van Eckerveld, M.	58
		Venturiello, S.M.	26, 72, 73
Oswald, I.	47	Verdier, M.	111
Pagani, P.	60	Verzoletti, M.L.	72
Panaitescu, D.	77, 78, 79	Victor, B.	101
Park, P.W.	35	Villegas-Sepulveda, N.	12
Pastusiak, K.	68, 119	Vittori, O.	82
Patrascu, I.V.	107	Wang, Z.Q.	25, 43, 44, 45, 120, 121
Paulauskas, V.	63		
Pavlovic, S.	116	Webster, P.	5, 6, 7, 23, 24, 32, 39
Petersen, E.	61, 62, 118	Wei, H.Y.	45, 120
Petrakos, M.	39	Wu, X.P.	13, 14, 15, 17
Petrovic, M.	107	Wu, Z.	28
Piaggi, S.	34	Xu, B.L.	44, 120
Picherot, M.	47	Xudong, Z.	88
Piergili-Fioretta, D.	82	Yan, D.	88
Potter, M.A.	110	Yang, J.	11
Pourquier, J.	119	Yang, Y.	11
Pozio, E.	1, 2, 4, 7, 8, 18, 31, 38, 60, 70, 75, 76, 98, 103, 119	Yera, H.	9
		Yepez-Mulia, L.	12, 41, 42, 51, 71
Proaño-Pérez, F.	101		
Prokou, M.	39	Yuan, L.H.	13, 14
Ramírez, M.	41	Zanginyan, A.	102
Reveles-Herández, G.	55, 56, 85, 86, 87, 105	Zarlenga, D.S.	1, 8, 10, 64, 98
		Zhan, B.	11
Ribicich, M.	29, 90, 111	Zhang, Y.L.	13, 14
Richardson, E.K.B.	110	Zhang, H.W.	44, 45
Rikula, U.	59	Zhang, D.	120, 121
Risti, J.	38	Zhu, X.	11

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