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Scientific Program

CONFERENCE VENUE
GRAND HOTEL NAPOCA

Monday, 26 August 2019	
14:00-19:00	**Registration**
13:00-15:00	**ICT Guidelines Committee Meeting**
16:00-17:30	**ICT Executive Committee Meeting**
19:00-21:00	**Welcome dinner**

Tuesday, 27 August 2019

08:00-16:30 • **Registration**

09:00-09:45 • **Opening Ceremony**

Section I: Phylogeny, taxonomy, and biology of *Trichinella* genus

Tuesday, 27 August 2019, 09:45-13:00

Interval	Presenter	Authors/Title
Session I	**Chairs: Benjamin Rosenthal, Zuzana Hurnikova**	
09:45-10:15	**Keynote**: **Dante Zarlenga**	**Zarlenga D.** **0092** Horizontal gene transfer of cyanase provides evidence for early associations between members of the Kingdom Plantae and the last common ancestor of *Trichinella* and *Trichuris*
10:15-10:30	**Ewa Bilska-Zając**	**Bilska-Zając E, Franssen F, Różycki M, Swart A, Karamon J, Sroka J, Zdybel J, Ziętek-Barszcz A, Cencek T.** **0013** Intraspecific genetic variation in *Trichinella spiralis* and *Trichinella britovi* populations circulating in different geographical regions of Poland
10:30-10:45	**Peter Thompson**	**Thompson PC, Bilska-Zajac E, Zarlenga DS, Liu M, Cencek T, Różycki M, Rosenthal BM.** **0080** Complete mitochondrial genomes and ribosomal DNA sequences of *Trichinella spiralis* indicate that the split between Asian and European populations happened prior to the rise of agriculture
10:45-11:00	**Fernando Fariña**	**Fariña FA, Pasqualetti MI, Ercole ME, Bessi C, Montalvo F, Vargas C, Krivokapich SJ, Ribicich MM.** **0030** Intestinal phase approach of *Trichinella patagoniensis* in balb/c mice
11:00-11:30	**Coffee break**	
Session II	**Chairs: Bretislav Koudela, Radu Blaga**	
11:30-11:45	**Benjamin Rosenthal**	**Rosenthal BM, Hecht BBL, Thompson PC.** **0067** A new method to reconstruct past population growth and decline suggests that, in both Europe and Asia, *Trichinella spiralis* has prospered and declined with wild boar

11:45-12:00	**Tingting Li**	**Tingting Li, Bin Tang, Haining Shi, Wenbao Zhang, Zhuangzhi Zhang, Jiaojiao Lin, Xiaolei Liu, Mingyuan Liu** **0082** Development of genome-wide-based polymorphic microsatellite markers and phylogenetic analysis of *Trichinella spiralis* in Chinese population
12:00-12:15	**Olga Rudneva**	**Rudneva OV, Andreyanov ON, Sidor EA.** **0071** Changes in the level of glycogen and the invasive ability of the *Trichinella nativa* larvae stored in natural conditions
12:15-12:30	**Sharma Rajnish**	**Sharma R, Thompson P, Hoberg EP, Scandrett B, Konecsni K, Harms NJ, Kukka PM, Jung TS, Elkin B, Mulders R, Larter NC, Branigan M, Pongracz J, Wagner B, Rosenthal B, Jenkins E.** **0074** Discovery of an undescribed species of *Trichinella* in northwestern Canada
12:30-12:45	**Wieslaw Kozek**	**Kozek W.** **0099** Life Cycle of *Trichinella spiralis* Revisited - With Morphological and Ultrastructural Correlations
12:45-13:00	**Anqi Wang**	**Wang A, Heckmann A, Caignard G, Vitour D, Liu M, Bruneau S, Boireau P, Vallée I, Karadjian G.** **0085** The New-Born Larvae stage specific serine protease NBL1 interacts with the host's cell Vimentin
13:00-14:30	**Lunch** **Poster Session**	
Posters	**Phylogeny, taxonomy, and biology of *Trichinella* genus** 1. **Karadjian G, Bahn P, Johne A, Gassiloud B, Py J-S, Mayer-Scholl A, Vallée I.** *Trichinella* species identification by MALDI-TOF (**0050**) 2. **Thompson PC, Hecht LBB, Rosenthal BM.** Distinct histories of population growth and decline can be inferred for the various species of *Trichinella*, as determined from patterns of heterozygosity in their genomes (**0079**) 3. **Xi Zhang, Lu Lu Han, Xiu Hong, Peng Jiang, Na Li, Xue Liu, Zhong Quan Wang, Jing Cui.** Genotyping and phylogenetic position of *Trichinella spiralis* isolates from different geographical locations in China (**0088**)	

Section II: Epidemiology of human and animal infection Tuesday, 27 August 2019, 14:30-16:45		
Session III	**Chairs: Mabel Ribicich, Gianluca Marucci**	
14:30-15:00	**<u>Keynote</u>:** **Samson Mukaratirwa**	**La Grange LJ, Mukaratirwa S.** **0095** Epidemiology of trichinellosis in Greater Kruger National Park, South Africa
15:00-15:15	**Ewa Bilska – Zając**	**Bilska – Zając E, La Rosa G, Pozio E, Różycki M, Cencek T.** **0011** Microsatellite analysis – the useful tool to track transmission of *Trichinella* spp.
15:15-15:30	**Zuzana Hurníková**	**Antolová D, Fecková M, Valentová D, Avdičová M, Hurníková Z.** **0096** Trichinellosis in Slovakia in last ten years, 2009-2018
15:30-15:45	**Ilaria Pascucci**	**Badagliacca P, Di Sabatino D, Cocco A, Romeo G, Salucci S, Tieri E, Salini R, Pascucci I.** **0003** Distribution of *Trichinella britovi* larval burden in muscular districts of naturally infected wild species in the Central Apennines, Italy.
15:45-16:00	**Zuzana Hurníková**	**Hurníková Z, Miterpáková M, Komorová P, Chovancová G.** **0041** 15 years from the first record of *Trichinella pseudospiralis* in Slovakia: What´s New?
16:00-16:15	**Olimpia Iacob**	**Iacob OC, Pașca SA, Bostănaru AC, Miron LD.** **0042** Influence of controlled freezing on larval viability by *Trichinella britovi* from wild boar meat
16:15-16:30	**Sasa Vasilev**	**Vasilev S, Mitic I, Plavsa D, Ilic N, Cvetkovic J, Sofronic-Milosavljevic L.** **0084** *Trichinella* infection in Serbia, from 2014 to 2018
16:30-16:45	**Radu Blaga**	**Blaga R.** **0104** A historical perspective of trichinellosis in Romania: the begining of the end?
16:45-17:30	• **Refreshing break**	
17:30-19:30	• **City Tour**	
19:30-21:00	• **Dinner in a traditional Romanian restaurant: "Roata" Restaurant**	

Wednesday, 28 August 2019		
Section II: Epidemiology of human and animal infection Wednesday, 28 August 2019, 09:00-10:45		
Interval	**Presenter**	**Authors/Title**
Session I	**Chairs: Bożena Moskwa, Ewa Bilska–Zając**	
09:00-09:30	**Keynote**: **Eduardo Pozio**	**Pozio E.** **0065** The impact of the climate change and human behavior on the biology and epidemiology of *Trichinella*
09:30-09:45	**Mabel Ribicich**	**Ribicich MM, Fariña FA, Aronowicz T, Ercole ME, Bessi C, Pasqualetti MI.** **0066** Trichinellosis scenarios in people, domestic and wild animals in South America
09:45-10:00	**Brad Scandrett**	**Scandrett B, Konecsni K.** **0073** *Trichinella* spp. in susceptible wildlife from swine-producing regions of Canada
10:00-10:15	**Břetislav Koudela**	**Koudela B, Harna J, Pijáček M.** **0053** Epidemiology and new trends in wild boar trichinellosis in the Czech Republic
10:15-10:30	**Bao-Quan Fu**	**Zhang NZ, Cong W, Jin QW, Li WH, Li TT, Liu YJ, Li L, Yan HB, Jia WZ, Fu BQ**. **0060** Isolation and Species Identification of *Trichinella* sp. from Farmed Minks (*Neovison vison*) in Shandong Province, China
10:30-10:45	**Anne Mayer-Scholl**	**Mayer-Scholl A, Wagner T, Staubach C, Schulze C, Nöckler K, Johne A, Selhorst T, Müller-Graf C.** **0057** The raccoon dog as reservoir and vector for *Trichinella* in Germany
10:45-11:15	**Coffee break**	
Section III: Human trichinellosis Wednesday, 28 August 2019, 11:15-12:45		
Session II	**Chairs: Francisco Bolás-Fernández / Violeta Briciu, Alessandra Ludovisi**	
11:15-11:45	**Keynote**: **Mihaela Lupşe**	**Lupse M, Flonta M, Rus M, Briciu V.** **0058** Human trichinellosis in Romania - a never ending story?

11:45-12:00	**Helene Yera**	**Caron Y, Bory Sotharith, Prum Sang Houn, Lim Sun Bun Hong, Vallée I, Sengdoeun Yi, Sovann Ly, Yera H.** **0018** First description of *Trichinella papuae* involved in an outbreak in central Kampong Thom province in Cambodia
12:00-12:15	**Helene Yera**	**Barruet R, Devez A, Dupouy-Camet J, Gely F, Karadjian G, Plavsa D, Chydériotis G, Vallée I, Sofronic-Milosavljevic L, Yera H.** **0004** Backyard pigs: a common source for a trichinellosis outbreak reported in France and Serbia in 2017
12:15-12:30	**Alessandra Ludovisi**	**Pozio E, Ludovisi A, Pezzotti P, Bruschi F, Gómez-Morales MA.** **0064** Trichinellosis in Italy from 2005-2016: a retrospective study based on the analysis of hospital discharge records
12:30-12:45	**Dalia Ashour**	**Eid RK, Ashour DS, Arafa MF, Essa EA, El Maghraby GM.** **0026** Enhanced oral bioavailability of albendazole against *Trichinella spiralis* infection by nanostructured lipid carriers
12:45-14:45	**Lunch; Poster Session**	
Posters	**Epidemiology of human and animal infection** 1. **Glawischnig W, Schöpf K.** *Trichinella* spp. findings in Austrian wildlife between 2011 - 2018 (**0034**) 2. **Bilska-Zając E, Różycki M, Chmurzyńska E, Antolak E, Gradziel-Krukowska K, Karamon J, Sroka J, Zdybel J, Cencek T.** First case of *Trichinella nativa* infection in wild boar in Central Europe - molecular characterization of the parasite (**0012**) 3. **Balić D, Dijanić T, Agičić M, Kaltenbrunner M, Mujić S, Hochegger R, Škrivanko M, Kozul K.** Croatia: Trichinellosis outbreak due to consumption of homemade smoked wild boar meat products (**0024**) 4. **Bilska – Zając E, Różycki M, Mayer-Scholl A, Nöckler K, Bahn P, Cencek T.** The results of MALDI-TOF MS investigations on protein profile of *Trichinella* isolates (**0009**) 5. **Boros Z, Ionică AM, Deak G, Mihalca AD, Györke A, Gherman CM, Cozma V.** New host recorded for *Trichinella britovi* in Romania: the European badger, *Meles meles* (**0014**)	

6. **Bilska – Zając E, Różycki M, Cencek T.**

The epidemiological situation of trichinellosis in Poland - past, present and future (**0010**)

7. **Cybulska A, Kornacka A, Moskwa B.**

The raccoon dog (*Nyctereutes procyonoides*) as a reservoir of *Trichinella britovi* in Poland (**0021**)

8. **Kołodziej-Sobocińska M, Hurníková Z, Miterpáková M, Zalewski A, Dvorožňáková E, Kowalczyk R.**

Occurrence of *Trichinella* spp. in carnivore community in Poland (**0052**)

9. **Winter S, Abate SD, Fariña FA, Pasqualetti MI, Ribicich MM.**

"Know to prevent" in northern Patagonia, Argentina (**0087**)

10. **Zhang NZ, Zhang XX, Li WH, Li TT, Jin QW, Liu YJ, Li L, Yan HB, Jia WZ, Cong W, Fu BQ.**

Detection of *Trichinella* spp. in Farmed Wild Boars (*Sus scrofa*) in Jilin Province, Northeast China (**0093**)

11. **Różycki M, Bilska-Zając E, Karamon J, Wiśniewski J, Krukowska KG, Cencek T.**

Trichinella in wild boar: analysis of long-term serological surveillance in Poland (**0068**)

12. **Pasqualetti MI, Fariña FA, Krivokapich SJ, Gatti GM, Daneri GA, Varela EA, Lucero S, Ercole ME, Bessi C, Winter M, Ribicich MM.**

Trichinella spiralis in *Otaria flavescens* from Patagonia, Argentina (**0097**)

13. **Grigoryan G, Aghayan SA, Gevorgyan H, Malkhasyan A, Vallée I, Karadjian G.**

The first report of *Trichinella britovi* in Armenia (**0037**)

14. **Anna Lundén.**

Trichinella in wildlife in Sweden 2007 - 2018 (**0108**)

Human trichinellosis

1. **Ammar NA, Karadjian G, Foulet F, Chouk R, Gaultier F, Ortonne N, Yera H, Botterel F.**

Trichinella spiralis stayed more than 30 years in human tongue (**0002**)

Genomics and proteomics

1. **Jing Ding, Bin Tang, Xuelin Wang, Haining Shi, Wenbao Zhang, Zhuangzhi Zhang, Jiaojiao Lin, Xiaolei Liu, Liu M.**

Excretion and secretion product of *Trichinella spiralis* can affect functions of neutrophils (**0048**)

2. **Grzelak S, Bień-Kalinowska J.**

Comparative analysis of excretory-secretory antigens of *Trichinella spiralis* and *T. britovi* adult worm by two-dimensional gel electrophoresis coupled with immunoblotting (**0039**)

	3. **Yang Wang, Bin Tang, Yulu Zhang, Haining Shi, Wenbao Zhang, Zhuangzhi Zhang, Jiaojiao Lin, Xiaolei Liu, Liu M.** iTRAQ-based differential proteomic analysis of excretory–secretory proteins of *Trichinella pseudospiralis* (**0091**) 4. **Ruo Dan Liu, Peng Jiang, Shao Rong Long, Xi Zhang, Zhong Quan Wang, Jing Cui** Screening and characterization of early diagnostic antigens from surface and ES proteins of *Trichinella spiralis* various developmental stages by immunoproteomics (**0072**)	

Section IV: Genomics and proteomics
Wednesday, 28 August 2019, 14:45-16:15

Session III	**Chairs: Pascal Boireau, Gregory Karadjian**	
14:45-15:15	**Keynote**: **Mingyuan Liu**	**Liu Mingyuan** **0102** Antigenic and functional genes in *Trichinella* spp. today
15:15-15:30	**Michał Gondek**	**Gondek M, Herosimczyk A, Knysz P, Ożgo M, Lepczyński A, Szkucik K.** **0036** Comparative proteomic analysis of serum from pigs experimentally infected with *Trichinella spiralis*, *Trichinella britovi* and *Trichinella pseudospiralis*
15:30-15:45	**Hua Nan Ren**	**Hua Nan Ren, Ruo Dan Liu, Kai Xia Guo, Yao Zhang, Shao Rong Long, Peng Jiang, Xi Zhang, Zhong Quan Wang, Jing Cui** **0040** Label-free quantitative proteomic analysis of molting-related proteins of *Trichinella spiralis* intestinal infective larvae
15:45-16:00	**Grégory Karadjian**	**Karadjian G, Heckmann A, Blanchard A, Grasteau A, Boireau P, Neveu C, Martin C, Vallée I.** **0051** Transient inactivation of *Trichinella spiralis* adults unc-63 by RNA interference
16:00-16:15	**Hu Xiaoxiang**	**Hu X, Liu X, Yang Y, Wang A, Shi H, Luo X, Jia W, Cai X, Vallee I, Boireau P, Bai X, Liu M.** **0089** The roles of serine protease-like protein from the new-born larvae stage of *Trichinella spiralis* in regulating collagen synthesis and differentiation on C2C12 myoblasts in vitro
16:15-17:00	• **Refreshing break**	
17:00-19:30	• **Turda Salt Mine trip**	
19:30-21:30	• **Dinner at the candle lights / wine tasting**	

Thursday, 29 August 2019

Section V: Legislation and control
Thursday, 29 August 2019, 09:00-10:15

Interval	Presenter	Authors/Title
Session I	**Chairs: Alvin Gajadhar, Walter Basso**	
09:00-09:30	**Keynote**: **Frits Franssen**	**Franssen F, Takumi K, van der Giessen J, Swart A.** **0031** Assessing the risk of human trichinellosis from pigs kept under controlled and non-controlled housing in Europe
09:30-09:45	**Annette Johne**	**Johne A, Gayda J, Nöckler K, Meyer D, Bandick N, Mayer-Scholl A.** **0049** Survival of *Trichinella spiralis* in cured meat products
09:45-10:00	**Milena Zivojinović**	**Zivojinović M, Dobrosavljevic I, Kulisic Z, Radojicic S, Boskovic T, Plavsic B, Vasilev S, Sofronic-Milosavljevic L.** **0094** Current status of implementation of the "One Health" concept in monitoring and control of *Trichinella* spp. Infections in Serbia
10:00-10:15	**Daoxiu Xu**	**Daoxiu Xu, Bin Tang, Haining Shi, Wenbao Zhang, Zhuangzhi Zhang, Jiaojiao Lin, Xiaolei Liu, Liu M.** **0023** Vaccination with DNase II recombinant protein against *Trichinella spiralis* infection in pigs
10:15-10:45	**Coffee break**	

Section VI: Anniversary celebration of ICT
Thursday, 29 August 2019, 10:45-13:00

Session II	**Chairman: Joke van der Giessen, Karsten Nöckler**	
10:45-11:30	**Dickson Despommier**	**0105** Urban Metamorphosis 2.0
11:30-12:00	**Alvin Gajadhar**	**0103** Contributions in control testing and post-harvest interventions for the management of *Trichinella* and trichinellosis.
12:00-12:30	**Jean Dupouy-Camet**	**0100** A French medical mission in Germany to study the trichinellosis outbreak of Emersleben (1883)
12:30-13:00	**Eduardo Pozio**	**0101** Scientific achievements of the last 60 years: From a single to a multispecies concept of the genus *Trichinella*
13:00-14:30	**Lunch; Poster Session**	

Posters	**Legislation and Control** 1. **Franssen F, Deng H, Swart A, Bonacic Marinovic A, Liu X, Liu M, van der Giessen J.** Inactivation of *Trichinella* muscle larvae at different time-temperature heating profiles (**0032**) 2. **Ercole ME, Bessi C, Pasqualetti MI, Ribicich MM, Aronowicz T, Montalvo F, Acerbo M, Fariña FA.** Gamma radiation effect on *Trichinella spiralis* and *Trichinella pseudospiralis* infected wild boar meat (**0028**) 3. **Agicic M, Balic D, Majic L, Kresic K, Skrivanko M.** *Trichinella* infection in humans and pigs in Croatia (1995-2014) - results of measures taken (**0001**) **Anniversary celebration of ICT** 1. **Marucci G, La Rosa G, Galati F, Interisano M, Tonanzi D, Gomez Morales MA, Ludovisi A, Amati M, Cherchi S, Possenti A, Rossi P, Pozio E.** The International Trichinella Reference Centre (ITRC): 30 years (1988-2018) of activity (**0056**) **Detection** 1. **Gajadhar A.** New reagent products for improved handling and dispensing of HCl and pepsin in the gold standard digestion method for the detection of *Trichinella* (**0033**) 2. **Lobanov V, Konecsni K, Scandrett B.** Comparison of the diagnostic performance of an in-house and commercial ES ELISA for the detection of *Trichinella* infection in pigs (**0055**) 3. **Bilgiç FI, Öztürk EA, Erdoğan DD, Korkmaz M, Gomez Morales MA.** Detection of Anti-*Trichinella* IgG In Dogs In Turkey-A Preliminary Study (**0008**) 4. **Gnjatovic M, Gomez-Morales MA, Gruden-Movsesijan A, Ilic N, Vasilev S, Sofronic-Milosavljevic L.** Evaluation of a novel competitive ELISA for detection of *Trichinella* infection in swine experimentaly infected with different species of *Trichinella* genus (**0098**)	

Section VII: Detection
Thursday, 29 August 2019, 14:30-16:30

Session III	**Chairs: Brad Scandrett, Pikka Jokelainen**	
14:30-15:00	**Keynote**: **Maria Angeles Gómez Morales**	**Gómez Morales MA, Merialdi G, Licata E, Della Casa G, Amati M, Cherchi S, Ramini M, Faeti V, Interisano M, Ludovisi A, Rugna G, Marucci G, Tonanzi D, Pozio E.** **0035** Relationship between anti-*Trichinella* IgG levels and muscle larvae in long lasting *Trichinella* infections in pigs

15:00-15:15	Alvin Gajadhar	**Buholzer P, Gajadhar A.** **0017** Validation of the PrioCHECK *Trichinella* AAD Kit for the detection of larvae in pork, and preliminary studies on horse meat and wildlife tissue
15:15-15:30	Chen Xi Hu	**Chen Xi Hu, Jie Zeng, Xin Yue, Peng Jiang, Shao Rong Long, Ruo Dan Liu, Xi Zhang, Zhong Quan Wang, Jing Cui** **0019** Molecular characterization of a *Trichinella spiralis* elastase-1 and its potential for serodiagnosis of trichinellosis
15:30-15:45	Richard Lagrimas	**Lagrimas RD, Gonzales RMC, Briones JCA.** **0054** Low *Trichinella* spp. (Railliet, 1895) Antibodies Detected in Domestic Pigs from Selected Slaughterhouses with Farm Risk Assessment in Bulacan, Philippines
15:45-16:00	Fernando Fariña	**Bessi C, Ercole ME, Fariña FA, Ribicich MM, Bonboni A, Acerbo M, Krivokapich SJ, Pasqualetti MI.** **0007** *Trichinella patagoniensis* in wild boars: a first approach
16:00-16:15	Walter Basso	**Basso W, Gottstein B, Frey CF** **0005** Evaluation of the "PrioCHECK *Trichinella* AAD kit" to detect *T. britovi*, *T. spiralis* and *T. pseudospiralis* in muscle tissue of domestic pigs by the automated digestion method Trichomatic-TM35
16:15-16:30	Aleksandra Cybulska	**Cybulska A, Kornacka A, Popiołek M, Bień-Kalinowska J, Moskwa B.** **0022** Immunoprevalence of *Trichinella* nematodes in raccoons (*Procyon lotor*) from the Czech Republic, Germany and Poland
16:30-17:30	• **Refreshing break**	
17:30-19:30	• **Visit to the Village Museum**	
19:30-21:30	• **Dinner: The Premier Restaurant**	

Friday, 30 August 2019

Section VII: Detection

Friday, 30 August 2019, 09:00-10:00

Interval	Presenter	Title/Authors
Session I	**Chairs: Patrizia Rossi, Anne Mayer-Scholl**	
09:00-09:15	Yan Liu	**Yan Liu, Xiaolei Liu, Yansong Li, Ning Xu, Yuying Yang, Liu L, Yu Zhou** **0090** Characterization of antigenic properties of a cystatin-like protein of *Trichinella spiralis* at its early invasion stage
09:15-09:30	Nan Wang	**Nan Wang, Bin Tang, Xuelin Wang, Haining Shi, Wenbao Zhang, Zhuangzhi Zhang, Jiaojiao Lin, Xiaolei Liu, Liu M.** **0086** Activity, infectivity and antibody dynamics response against *Trichinella spiralis* in experimentally infected pigs
09:30-09:45	András József Laki	**Szélig AG, Hartdégen M, Iván K, Kucsera I, Laki AJ.** **0078** Filtration of *Trichinella* larvae using a microfluidic device
09:45-10:00	Mirosław Różycki	**Różycki M, Bilska-Zając E, Karamon J, Wiśniewski J, Nowicki M, Bogdan J, Cencek T.** **0069** Distribution of *Trichinella spiralis* larvae in muscle of naturally infected pigs

Section VIII: Pathogenesis and immunology

Friday, 30 August 2019, 10:00-13:30

Session II	Chairs: Isabelle Vallee, Mingyuan Liu	
10:00-10:30	**Keynote**: **Ljiljana Sofronic-Milosavljevic**	**Bruschi F, Sofronic-Milosavljevic L, Gruden-Movesijan A, Pinto B, Ilic N.** **0016** *Trichinella spiralis* excretory-secretory products decrease the level and activity of matrix metalloproteinase 9 (MMP-9) on the model of experimental autoimmune encephalomyelitis in DA rats

10:30-10:45	Natasa Ilic	**Cvetkovic J, Ilic N, Gruden-Movsesijan A, Tomic S, Mitic N, Pinelli E, Sofronic-Milosavljevic L.** **0020** DC-SIGN, TLR2 and TLR4 signalling by *Trichinella spiralis* excretory /secretory antigens is required for the induction of tolerogenic human DC
10:45-11:00	Jia Xu	**Jia Xu, Ruo Dan Liu, Shao Rong Long, Peng Jiang, Xi Zhang, Zhong Quan Wang, Jing Cui** **0044** Characterization of a chymotrypsin-like enzyme from *Trichinella spiralis* and its facilitation on larval penetration of host's intestinal epithelial cells
11:00-11:15	Emília Dvorožňáková	**Dvorožňáková E, Vargová M, Lauková A, Revajová V.** **0025** Dynamics of lymphocyte subpopulations in the small intestine of mice treated with probiotic bacteria and infected with *Trichinella spiralis*
11:15-11:30	Qi-Wang Jin	**Jin QW, Zhang NZ, Li WH, Li TT, Liu YJ, Li L, Yan HB, Jia WZ, Fu BQ** **0045** Regulation of Mouse Type 2 Immune Response Induced by the Thioredoxin Peroxidase-2 （TPX2） from *Trichinella spiralis*
11:30-11:45	**Coffee break**	
Session III	**Chairs: Mirosław Różycki, Natasa Ilic**	
11:45-12:00	Olga Rudneva	**Berezhko VK, Novik TS, Kamvshnikov OY, Danilova TI, Rudneva OV, Napisanova LA, Koveshnikova EI, Thakakhova AA.** **0006** Evaluation of *Trichinella spiralis* larvae extract as an inhibitor of antiproliferative effect on human breast cancer cell culture - MCF-7
12:00-12:15	Alisa Gruden-Movsesijan	**Gruden-Movsesijan A, Tomic S, Ilic N, Glamoclija S, Todorovic A, Vasilev S, Stojanovic D, Miljkovic D, Sofronic-Milosavljevic L.** **0043** Shooting the autoimmunity by *Trichinella* molecules
12:15-12:30	Ning Xu	**Xu N, Liu X, Yong Yang, Haining Shi, Xuenong Luo, Wanzhong Jia, Xuepeng Cai, Vallee I, Boireau P, Liu M, Xue Bai** **0061** Serine protease inhibitor of *Trichinella spiralis* induced a process of early anti-inflammatory immune response dependent on macrophages alternative activation *in vivo*

12:30-12:45	Peng Jiang	**Peng Jiang, Shu Wei Yan, Yan Yan Song, Jia Xu, Shao Rong Long, Ruo Dan Liu, Xi Zhang, Jing Cui, Zhong Quan Wang** **0062** Interaction between *Trichinella spiralis* enolase and human plasminogen
12:45-13:00	Xuemin Jin	**Jin X, Yang Y, Liu X, Haining Shi, Xuenong Luo, Wanzhong Jia, Xuepeng Cai, Vallee I, Boireau P, Xue Bai, Liu M.** **0046** The NLRP3 play a critical role in development of Th2 cell-mediated protective immune response against *Trichinella spiralis* infection
13:00-13:15	Anna Stachyra	**Stachyra A, Basałaj K, Zawistowska-Deniziak A, Grzelak S, Bień-Kalinowska J.** **0077** Immunogenicity of recombinant multi-cystatin-like domain protein from *Trichinella britovi*: In Vivo study in mouse model
13:15-13:30	Shu Wei Yan	**Shu Wei Yan, Peng Jiang, Yan Yan Song, Jia Xu, Shao Rong Long, Ruo Dan Liu, Xi Zhang, Jing Cui, Zhong Quan Wang** **0075** Characterization of *Trichinella spiralis* cathepsin X and its role in larval invasion of intestinal epithelial cells
13:30-15:00	**Lunch** **Poster Session**	
Posters	**Pathogenesis and immunology** 1. **Jing Ding, Bin Tang, Xuelin Wang, Haining Shi, Wenbao Zhang, Zhuangzhi Zhang, Jiaojiao Lin, Xiaolei Liu, Liu M.** *Trichinella spiralis* can induce neutrophils to release NETs (**0047**) 2. **Song Yining, Jing Xu, Xiaolei Liu, Yong Yang, Jianda Pang, Xinrui Wang, Mingchuan Yu, Mingyuan Liu, Shumin Sun.** Regulation of host immune cells and cytokines expression induced by *Trichinella spiralis* infection (**0076**) 3. **El Gendy DI, Othman AA, Hasby Saad MA, Soliman NA, Mwafy SE.** Resveratrol reduces oxidative damage and inflammation in mice infected with *Trichinella spiralis* (**0027**) 4. **Vargová M, Bucková B, Hrčková G, Lauková A, Dvorožňáková E.** Cytokine gene expression in the gut and in the spleen of mice modulated by probiotic bacteria and *Trichinella spiralis* infection (**0083**)	

	5. **Li TT, Zhang NZ, Li WH, Jin QW, Liu YJ, Li L, Yan HB, Jia WZ, Fu BQ.** Experimental Infection with *Trichinella spiralis* Tibet strain in Hezuo Pig (**0081**) 6. **Rudneva OV, Napisanova LA, Berezhko VK.** Evaluation of the protective effect of various immunostimulating drugs in experimental trichinosis of mice (**0070**) 7. **Movsesyan SO, Petrosyan RA, Nikogosyan MA, Terenina NB, Gómez-Morales MA, Voronin MV.** Plasmagenesis dynamics in experimental *Trichinella* infection (**0059**) 8. **Piaggi S, Salvetti A, Mazzoni S, Gomez-Morales MA, Pinto B, Bruschi F.** Glutathione-S-transferase omega 1: a possible role in nurse cell development (**0063**) 9. **Fan Yang, Da Qi Yang, Yan Yan Song, Kai Xia Guo, Ya Lan Li, Shao Rong Long, Peng Jiang, Zhong Quan Wang, Jing Cui** *In vitro* silencing of serine protease inhibitor suppresses *Trichinella spiralis* invasion, development and fecundity (**0029**)
Section IX: Student Research Awards Friday, 30 August 2019, 15:00-16:00	
Session IV	**Chairs: Joke van der Giessen, Karsten Nöckler**
15:00-16:00	**1. Rajnish Sharma***, Konecsni K, Scandrett B, Thompson P, Hoberg EP, Bouchard E, Buhler K, Harms NJ, Kukka PM, Jung TS, Elkin B, Mulders R, Larter NS, Branigan M, Pongracz J, Wagner B, Fenton H, Rosenthal B, Jenkins E. **0106** Distribution and genetic diversity of *Trichinella* in Canadian wildlife: A previously undescribed species (T13) and an unexpected discovery of *T. spiralis* **2. Bessi C***, Ercole ME, Fariña FA, Ribicich MM, Bonboni A, Montalvo F, Acerbo M, Krivokapich SJ, Pasqualetti MI. **0107** Experimental study in wild boars infected with *Trichinella patagoniensis*, *T. pseudospiralis* and *T. spiralis*
16:00-17:00	• **The second meeting of the ICT Executive Committee**
17:00-18:30	• **ICT Business Meeting and Closing Remarks**
20:00-24:00	**Gala Dinner**

SUMMARY

PHYLOGENY, TAXONOMY, AND BIOLOGY OF *TRICHINELLA* GENUS

ORAL PRESENTATIONS

0092 Horizontal gene transfer of cyanase provides evidence for early associations between members of the Kingdom Plantae and the last common ancestor of *Trichinella* and *Trichuris*

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Parasitism among nematodes has occurred in multiple, independent events. Deciphering processes that drive species diversity and adaptation are keys to understanding parasitism and advancing control strategies. Earlier studies were put forth on morphological and physiological aspects of parasitism and adaptation in nematodes; however, data is now coming available to investigate adaptation, host switching and parasitism at the genomic level. Evidence has been advanced showing that horizontal gene transfer (HGT) has played an important role in the evolution of nematodes. In a database search of cyanase genes which are typically present only in plants, bacteria and to a lesser extent fungi, we found more than 35 members of the Phylum Nematoda that also harbored this gene; however, none were found in free-living worms and none were present in organisms of the crown clade. Cloning and expressing the gene from *Trichinella spiralis*, a clade I organism, produced a protein capable of bicarbonate-dependent degradation of cyanate to ammonia and carbon dioxide in vitro. Immunological studies showed multimeric forms of the native protein in crude worm extracts of *T. spiralis* muscle larvae with a predilection site in the worm hypodermis. Phylogenetic analyses showed that encoded proteins from the clade I organisms *Trichinella* spp., *Trichuris* spp., and *Soboliphyme baturini*, (Subclass: Dorylaimia) formed a large, well-supported monophyletic clade with plant cyanases whereas all

cyanases found within the Subclass Chromadoria were monophyletic with those of bacterial origins. These results are consistent with: 1) independent HGT of the cyanase gene within parasitic nematodes but from different Kingdoms; 2) functional integration of the gene and encoded protein into the biology of *T. spiralis*; 3) acquisition within the Dorylaimia occurring over 400 million years ago prior to the divergence of the Trichinellida and Dioctophymatida, and 4) early free-living ancestors of the genus *Trichinella* having had an association with plants.

0080 Complete mitochondrial genomes and ribosomal DNA sequences of *Trichinella spiralis* indicate that the split between Asian and European populations happened prior to the rise of agriculture

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Trichinella spiralis has origins in Asia with subsequent spread to the rest of the world. Estimates of genetic diversity in European *T. spiralis* isolates indicated that the parasite went through a dramatic genetic bottleneck somewhere in its history. One hypothesis argued that this genetic bottleneck was associated with pig domestication and the transport of a limited number of *T. spiralis* infected pig hosts from Asian centers of domestication. In order to explore this hypothesis, we generated complete mitochondrial genomes and ribosomal DNAs from 22 European *T. spiralis* isolates, six North American isolates and nine Chinese samples using next-generation sequencing. A total of 13,858 base pairs of mitochondrial DNA and 7431 nucleotides of ribosomal

sequence were aligned and subjected to phylogenetic analysis using other *Trichinella* species as outgroups. North American and European isolates were tightly clustered within a single western clade. All Chinese *T. spiralis* were placed within a well-supported clade that was sister to the western clade. These results indicate that European *T. spiralis* does not directly descend from Chinese parasite populations. Furthermore, the amount of nucleotide divergence between the two clades is consistent with division prior to domestication of pigs. Over evolutionary time periods, Chinese and European *T. spiralis* were likely maintained as separate populations in wild boar that were separated by geographic features. The genetic bottleneck observed in European *T. spiralis* is not the result of a founder effect from a small number of parasites imported from China, but rather likely derives from the domestication of a small number of wild boar in Europe.

0030 Intestinal phase approach of *Trichinella patagoniensis* in balb/c mice

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The gut constitutes the initial place of contact of *Trichinella* with its host. The duration of the parasitism and the number of NBL produced by females in the intestines determines the extension of the disease. The aim of the present research was to study different aspects concerning the intestinal phase of *T. patagoniensis* in BALB/c mice. 128 female BALB/c mice were divided into two groups of 64 mice and were inoculated PO with 500 L1 larvae of *T. patagoniensis* and 500 L1 larvae of *T. spiralis* respectively. Euthanasia

was performed on days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 18, 20 y 25 pi. Then, adult worm recovery from intestines were performed. Samples from small intestine were cut and stained with hematoxylin and eosin. Systemic cytokine profile was evaluated during the intestinal phase of infection of *T. patagoniensis* from sera of animals euthanized on days 0, 2, 4, 6 and 9 p.i. using a mouse Th1/Th2/Th17 cytometric bead array kit (BD Biosciences, San Jose, CA). One-way ANOVA was performed followed by the Bonferroni's multiple comparison test. Intestinal adult worm recovery of *T. patagoniensis* took place until day 17. Intestinal adult worm recovery of *T. spiralis* took place until day 25. The recovery of adult worms resulted statistically different among day 9 on and day 1 and among day 10 on and day 1 for the treatment *T. spiralis* and *T. patagoniensis* respectively (p-value < 0.05). Hyperemia, mucosal oedema and inflammatory infiltrate was observed from day 1 pi in both *T. patagoniensis* and *T. spiralis* infected animals while hyperplasia of Peyer's patches was presented from day 20 pi. IFN-γ values progressively increase after the inoculation with *Trichinella* species until reaching a peak at 9 dpi in animals infected with *T. spiralis* and *T. patagoniensis*. The concentration of IL-10 reached a peak at 4 dpi in animals belonging to both experimental groups. The level of TNF showed a slight increased 3 dpi. IL-2, IL-4, IL-6 and IL-17 showed no significant variation during the study period. Although histopathologic changes of intestines and systemic cytokine profile shown a similar pattern in both *Trichinella* species, the duration of the intestinal phase evaluated as the time pi adult worms were able to remain in gut of BALB/c mice was shorter for *T. patagoniensis* than *T. spiralis*.

The present research was approved by the Committee for the Use and Care of Laboratory Animals (CICUAL) of the Facultad de Ciencias Veterinarias, University of Buenos Aires, under permit number 2014/01.

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0067 A new method to reconstruct past population growth and decline suggests that, in both Europe and Asia, *Trichinella spiralis* has prospered and declined with wild boar

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Host movements and interactions define parasite population dynamics, but controversy surrounds the longevity of host-parasite relationships. Host switching may lead to temporary interdependency such that parasite evolution is not bound by a single host. Because of its remarkable reproduction in swine, *Trichinella spiralis* is thought to have been dependent on wild boar (*Sus scrofa*) for much of its history. When considering the temporal durability of this relationship, it would be useful to understand whether *T. spiralis* and wild boar host populations have grown and contracted in concert. We devised methods to compare demographic histories, derived from genomic data. *Trichinella spiralis* and wild boar genomes from European derived isolates and Chinese isolates were assembled from sequence read archives stored in public databases and from novel sequencing projects with careful efforts to document heterozygous sites within each genome. The distribution of heterozygous bases across each genome was subjected to analysis using the Pairwise Sequentially Markovian Coalescent (PSMC) model to reveal demographic size through time for each sample. Pairwise comparisons of host and parasite demographic curves were examined, and a curve-fit metric was calculated based on the timing of population increase, decrease, or stasis over thousands of years. The magnitude of the curve-fit metric was used to evaluate whether host and parasite population growth histories were demonstrably parallel, implying a continued association through time. Across the most recent 120,000 years, demographic histories of *T. spiralis* and wild boar were remarkably similar and had much better curve-fit metrics than comparisons with randomized growth curves or growth curves from unrelated systems (humans, potatoes, or coral). Furthermore, the

growth history of *T. spiralis* proved regionally-specific, paralleling distinctive growth histories for wild boar in Asia and Europe. Comparative demography based on genomic sequences has established that *T. spiralis* populations have grown and declined in concert with wild boar populations in both Europe and Asia and provides a tool to understand many host-parasite relationships.

0082 Development of genome-wide-based polymorphic microsatellite markers and phylogenetic analysis of *Trichinella spiralis* in Chinese population

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Trichinella species are ovoviviparous parasitic nematodes which infect wildlife and domestic animals show a global geographic distribution. These

foodborne zoonotic parasites are responsible for the disease Trichinellosis and of great economical and healthy importance. *T. spiralis* is the most common species in China, which is considered to be relatively more polymorphic located in East Asia particularly. In this study, shotgun genomic sequencing data have been used to develop new microsatellite loci for the *T. spiralis* by using MISA. A total of 93140 microsatellites were identified from 9267 contigs of *T. spiralis* genome. Finally, 16 polymorphic loci were selected for primer validation based on population of *T. spiralis* from China. Allele numbers varied from 7 to 19 with an average value of 11.25 per locus. The observed heterozygosity (Ho) and expected heterozygosity (He) ranged from 0.250 to 0.600 and 0.793 to 0.918, respectively. Furthermore, the polymorphism information content (PIC) values ranged from 0.770 to 0.913 with an average value of 0.839. Ten out of the sixteen loci were successfully amplified in all twelve species through the cross amplification. Phylogenetic analysis of 10 isolates from different regions of China indicated that the Yunnan isolate was likely to diverge early in the history, may be the origin of other geographic strains. This study provides basic information of *T. spiralis* microsatellites, and the genome-wide markers development may be a useful tool for the genetic study of *Trichinella* species.

Keywords: *Trichinella spiralis*, Microsatellite, MISA, cross amplification, phylogenetic analysis

0071 Changes in the level of glycogen and the invasive ability of the *Trichinella nativa* larvae stored in natural conditions

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Trichinosis - helminthic disease of humans and animals, belonging to the group of dangerous helminth infections. *Trichinella* show predominantly

anoxybiotic nature of metabolism, their main storage substance is glycogen, which they accumulate in significant quantities at the muscular stage of development. Under the influence of negative temperatures the *Trichinella* larvae intensively spend glycogen and neutral fats, the mobilization of energy resources to ensure the basal metabolism continues until energy sources are reduced to a critical level. In this study, we established the concentration of glycogen and the invasive activity of *Trichinella nativa* larvae under the influence of negative temperatures on them.

Trichinella larvae of the species *T. nativa* were used in the studies. Laboratory rats (*Vistar*) were infected oral at a dose of 5 larvae per 1 gram of body weight and kept in vivarium conditions. After 9 months, the animals were subjected to drug euthanasia. Carcasses of animals laid in containers under the snow cover in the natural conditions of the hunting economy of the Ryazan region of Russia. Monthly (from January to April 2017) we monitored larvae viability and glycogen level. At the end of the experiment (april - month), the highlighted larvae were to the study of a bioassay in C57BL on 10 mice.

For the quantitative determination of glycogen content in larvae, a modified method was used, based on iodine staining, determination of the optical density with a refractometer, and construction of a measurement graph.

As a result of the research, it was found that the viability index of *Trichinella* larvae kept in vivo for 3 months in the muscle tissue of laboratory rats remained high (more than 90%). The glycogen level in terms of one larva of the helminth was 0.041 (in February); 0.032 (in March) and 0.014 (in April) mcg. In the control, the glycogen level index ranged from 0.047 to 0.043 μg. The survival rate of larvae was 5.3% in the experiment and 18.2% in the control. The invasive ability of the stored larvae was different. Of the three laboratory mice, one animal was infected (33.3%). In the control group, all animals were infected.

Thus, in winter, under the influence of negative temperatures, the viability index of the helminth larvae does not change. A decrease in glycogen concentration in *Trichinella nativa* larvae is noted, which affects the intensity of their metabolism.

0099 Life Cycle of *Trichinella spiralis* Revisited - With Morphological and Ultrastructural Correlations

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Application of molecular biology techniques to elucidate the many aspects of the complex host-parasite relationships that occur during infections by *Trichinella* spp. warrants a review of the life cycle to better understand the structure and biology of each stage of these fascinating parasites. We have examined, using light, scanning and transmission electron microscopy and soft X-ray microscopy, *T. spiralis* newborn larvae, muscle larvae and adult worms developing in the intestine, to elucidate their structure and formation, with emphasis on the cuticle formation, bacillary bands, stichosome, sensory structures, development of genitalia and the reproductive capacity. The newborn larva, intestinal stages and adults have a similar cuticular structure characterized by superficial horizontal ridges, internal radial striations and a horizontal bar in the apex of each ridge. Bacillary bands, containing patent pores, are present in the lateral chords of all stages except the muscle larvae. The cuticle of the muscle larva is modified into a thick, fairly homogenous, essentially two-layered structure with annular striations and without patent pores of the bacillary band. Newborn larvae have a functional stichosome containing secretory granules in at least ten stichocytes which persist in the stichosome of the muscle larva for at least ten days as newer generation of stichocytes continue to develop to form the mature stichosome of the muscle larva. Axonal terminations, observed as modified cilia, are present in the amphids, and in other receptors in the cephalic space, in some cells of the bacillary band, and at the end of copulatory appendage. The gender of the mature muscle larva can be identified early during intramuscular development by the length of the rectum and also, in mature larvae, by the primordia of the uterus and testis, and by the vaginal plate. During intestinal phase, both primordia unite with their respective end organ to form a patent duct. Maturation of oocytes and spermatocytes occurs at the same time as the

genital primordia extend to join to their respective end organ. After approximately 30 hours of development in the intestine, the larvae attain sexual maturity and can mate. The ability to identify the gender of muscle larvae provided the opportunity to determine, in Swiss mice, the reproductive capacity of each gender. The results of paired infections: 1 male (M) + 1 female (F), 1M+10F and 10M+1F, suggested that, in mice, the insemination capacity of the male is about 300 sperm/mating and it can produce more than 2,000 spermatozoa. The female can produce more than 3,000 oocytes and can be inseminated several times during its life period. The agents responsible for the transformation of the contractile elements of the infected muscle cell into smooth endoplasmic reticulum, are yet to be identified. This transformation may be initiated by some secretory components of the stichosomes, neurosecretions from the amphids, potential secretions of the bacillary bands or by excretions from the intestine. The life cycle of *T. spiralis* spp. is very complicated and calls for judicious selection of research materials to use in each experiment. The most appropriate protocols may indicate that the use of monosexual infections, testing single larva of defined gender, or female worms, collected from the intestine during the 24 hour period after infection to eliminate potential contamination with male sperm elements, will yield the best results.

POSTER PRESENTATIONS

0079 Distinct histories of population growth and decline can be inferred for the various species of *Trichinella,* as determined from patterns of heterozygosity in their genomes

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The genus *Trichinella* has an array of species that have differentiated through geographic isolation and some specialization for certain hosts. One aspect of the evolution of species is the effective population size, which can be thought of as the smallest population that would maintain all of the genetic variation present in the population. This effective population size can change over time resulting in loss of genetic variation when populations are small or gain as a population grows and new mutations are maintained. The results of these demographic changes are recorded in the genome as blocks of history which can be recombined through sexual reproduction and crossing over. By sequencing genomes, differences between maternal and paternal alleles are revealed as heterozygotes and their density is an indication of age of any particular DNA segment. By integrating across the entire genome, segments of different ages can be converted to effective population size based on their frequency in relation to a null distribution of how genomes should recombine over time. The end result is a demographic curve showing the growth, stasis, and decline of populations over time. We sequenced six species (*T. spiralis*, *T. nativa*, *T. britovi*, *T. pseudospiralis*, *T. murrelli*, and *T. nelsoni*) and one genotype (T6) in order to examine historic population sizes for each. Based on over 40 million base pairs of sequence from each, we show that the histories of these species are varied and must be dependent on factors specific to the ecology of each.

EPIDEMIOLOGY OF HUMAN AND ANIMAL INFECTION

ORAL PRESENTATIONS

0095 Epidemiology of trichinellosis in Greater Kruger National Park, South Africa

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Background

Knowledge on the development of changes influencing the infectivity, epidemiology and survival of *Trichinella* spp. in different climatological environments is important. This knowledge allows for the elucidation of epidemiology of *Trichinella* infections and the prediction of probable host-parasite cycles within specific ecological niches. The recent identification of new host species infected with three *Trichinella* taxa within the Greater Kruger National Park (GKNP) of South Africa prompted a revision of previously published hypothetical life cycles for these species. Unravelling the enigmatic epidemiology of these potentially zoonotic species from the genus *Trichinella* is important from a public health perspective as it may aid in establishing not only the potential risk for human infection but ultimately proper control and prevention measures.

Objective(s)

To illustrate and describe the hypothetical life cycles of *Trichinella* spp endemic in the GKNP of South Africa using data gathered from surveillance studies spanning the period 1964-2016.

Method and Materials

The hypothesized life cycles were established based on the epidemiological factors and prevalence data gathered from both the GKNP and

similar wildlife protected areas in Africa where the same host- and parasite species are known to occur.

Results

Results showed that *T. zimbabwensis* is the most prevalent, and also infects the widest host range of all the *Trichinella* species isolated thus far from the GKNP. This suggest the general knowledge and perceptions of interspecies predation and scavenging among terrestrial- and aquatic predators to be marginal. Successful incursion from the sylvatic cycle and the subsequent maintenance of the flow of parasites between sylvatic, synanthropic and domestic environments relies on parasite and ecological characteristics, human behaviour and availability of synanthropes. This ultimately results in unique life cycles for each taxon within a specific ecological niche.

Conclusions

The anecdotal nature of some of the reports and data confirms the need for more intense epidemiological surveillance in the rest of South Africa and continued efforts to unravel the epidemiology of *Trichinella* spp in this unique and diverse protected landscape.

0096 Trichinellosis in Slovakia in last ten years, 2009 – 2018

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In Slovakia, human and animal trichinellosis is constantly present in sylvatic cycle with several sporadic human outbreaks registered since 1930´s.

The aim of the study was to find out the trends in epidemiological situation in people and animals in last ten years in Slovakia.

Altogether, 29 human cases were reported to Public Health Authority of the Slovak Republic between 2009 and 2018. The number of cases varied between 0 and 13 per year, with minimum (none case) in 2009 and 2018 and maximum (13 cases) in 2011. Except one small family epidemic with three affected persons at the turn of 2011 and 2012, all reported cases were individual. Epidemiological anamnesis was documented in 10 cases, 8 patients stated consumption of wild boar meat and 2 people consumed pork.

Within the epidemiological survey performed at Institute of Parasitology SAS, 825 human serum samples were examined serologically by ELISA method. Positivity was recorded in three sera (0.36%); one positive person was a hunter, one worked as a veterinarian in the dog shelter, and the third person belonged to group of common population. Following Western Blot analysis confirmed seropositivity in two samples (2.42%), serum from a hunter and veterinarian.

Data about the trichinellosis in red foxes (*Vulpes vulpes*), wild boars (*Sus scrofa*) and brown bears (*Ursus arctos*) were reported to State veterinary and Food Institute. Since 2009, 2,295 red foxes were examined. The prevalence rate varied between 2.84% (6/211) in 2017 to 20.88% (38/182) in 2011. Although the number of examined wild boars was much higher (more than 165,000), their positivity was lower, ranging between 0.02% in 2017 to 0.07% in 2012 and 2013. Similarly, low positivity (1.37%) was recorded in 73 brown bears examined between 2014 and 2018. Within the compulsory monitoring of trichinellosis in domestic pigs none positive animal (0.0%) was recorded between 2009 and 2018. *Trichinella britovi* was predominant species (69.44%) recorded in wildlife, followed by *T. spiralis* (1.39%) and *T. pseudospiralis* (0.35%). In 28.82% positive animals, only *Trichinella* spp. was reported.

The study confirmed constant persistence of *Trichinella* spp. in wildlife in Slovakia with the red foxes being the main reservoir animals. As in the past, *Trichinella britovi* has remained predominant species; while *T. spiralis* and *T. pseudospiralis* occur only sporadically. Although no positive domestic animal was recorded during monitored period, almost annual occurrence of human cases suggests that the risk of human infection outbreaks in Slovakia still persists.

0003 Distribution of *Trichinella britovi* larval burden in muscular districts of naturally infected wild species in the Central Apennines, Italy

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In the Apennines regions, *T. britovi* has been mainly reported in wolf (*Canis lupus*) and red fox (*Vulpes vulpes*) and sporadically in wild boar (*Sus scrofa*) and Mustelidae.

In this study, we update the apparent prevalence (AP) of *T. britovi* infection in wildlife in the Abruzzi region in 2015-2018 period. Moreover, we studied the distribution of larvae in the muscular districts of infected animals to identify a representative muscle or group of muscles to be tested in order to predict the total number of larvae.

For this purpose, among positive animals, we randomly selected seven wolves and 5 foxes. We divided each body in 20 muscular districts, individually sampled, weighted and digested to detect the larvae per gram (LPG) and to estimate the larval burden per single muscular district (SLB) and the global larval burden per animal (GLB).

The AP observed in wolf was 28.3% (IC 95%: 21-37), in red fox 8.0% (IC 95%: 5.5-11.4), in wild boars 0.03% (IC 95%: 0-0.1), and in Mustelidae 1.96% (IC 95%: 0.6-6.8), confirming previous published data. The mean GLBs was 29896 (min 6078,3 max 62323,5) in wolf and 7934,7 (min 1813,5 max 13045,6) in fox. The average of LPGs was 1,05 (σ=0.71) in wolf and 1,65 (σ=1,05) in fox.

No statistically significant difference was observed between wolves and foxes regarding the SLB, GLB and the LPG. On average, the diaphragm in the wolf (LPG mean = 5,91), and the lower part of the left forelimb in the red fox (LPG mean = 7,57) showed the highest LPG.

Several linear regression models were performed to establish the ability of SLB to predict the GLB of animals. In foxes almost all muscular districts were significantly predictive (all R^2> 0.80) of the total number of larvae; whereas, in wolf were less than half. Notably, the higher R^2 for the SLB were found in the back in wolf (r^2:0.97) and in the upper part of right forelimb in the red fox (r^2:0.99).

The LPG of these muscular districts could be candidate to estimate the total number of larvae in the relative carcass.

In spite of the limited number of animal tested and the high variability observed, our study aims to be propaedeutic to estimate the larval biomass in fox and wolf population, as maintaining hosts, to better understand the trend of infection by *T. britovi* in wildlife in the Apennines.

0041 15 years from the first record of *Trichinella pseudospiralis* in Slovakia: What´s New?

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The non-encapsulated species *Trichinella pseudospiralis* is considered a cosmopolitan zoonotic parasite. The species is the only of the genus capable of infecting birds as well as mammals, including humans. The presence of the parasite in the Central Europe was for the first time recorded in 2003 in Eastern Slovakia. The first focus of *T. pseudospiralis* was documented in pigs, rats, and a cat and from a pig breeding farm in Eastern Slovakia. In following years the parasite was found in co-infection with *T. britovi* in wild boars and red foxes from the same region. Molecular analyses revealed distinctive genetic relationship of Slovak isolate with those from Finland and Sweden, suggesting the potential role of migratory birds of prey in transmission of the parasite. That was the reason why we focused our research on birds of prey from Slovakia. During 2006 – 2018 based on the special permit of the Ministry of Environment SR No. 6467 we

collected 360 pectoral muscle samples from carnivorous and omnivorous birds from Slovakia. Individual muscle samples were examined for the presence of *Trichinella* larvae by artificial digestion according to standard methods. 2000). The PCR was preformed according to protocol designed by Pozio and La Rosa (2003). The infection was diagnosed in two Common kestrels, one Peregrine falcon and one Golden eagle. All infected birds originated from eastern part of Slovakia. These results indicate that the parasite is already present in the environment of this part of Slovakia, as no of the infected birds belongs to regular migrants and most likely contracted the infection in their residence area. In 2017, the mandatory examination of wild boars revealed one individual from central part of territory being infected with *T. pseudospiralis,* what confirms that the parasite has already established in Slovakia.

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0042 Influence of controlled freezing on larval viability by *Trichinella britovi* from wild boar meat

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Purpose: Testing the viability of *Trichinella britovi* larvae from frozen wild boar meat controlled at different temperatures using a murine experimental model.

Material and Method: Muscle tissue parasitized with *T. britovi* larvae originated from a boar shot in the hunting season (December 2018) in the Vaslui area. From the highly parasitic muscles (diaphragm, tongue, intercostal muscles) were made medium samples of 50 g, which were deposited simultaneously in different temperature-controlled freezers where they were maintained for 56

days, as follows: -18°C (sample 1); -20°C (sample 2); -29°C (sample 3); -40°C (sample 4). Defrosting of the samples was done progressively at +4°C, and the larvae were extracted by artificial digestion and maintained in physiological saline until further use. The larvae from each sample were counted by the Euzeby method and administered by gavage to BALB/c mice at a daily dose of 60 larvae/mouse, two consecutive days to test their infective capacity. For this purpose, five batches of five mice, respectively four experimental batches and a control batch were formed. The experiment lasted 56 days and was completed by euthanasia of the mice. Following the necropsy examination, 5 fragments of muscle tissue (diaphragm, abdominal muscles, tongue, anterior leg muscles, dorsal muscles), were taken from each mouse, which were subsequently processed by the paraffin inclusion method, cut at 5 μm and stained by the Masson tricromatic method. Confirmation of the presence of larvae belonging to the *T. britovi* species was accomplished by multiplex PCR.

Results. The histopathological examination of muscle tissue taken from mice did not reveal trichinelic cysts in the experimental groups.

Conclusion: The low temperatures (-18°C; -20°C; -20°C; -40°C), applied for eight weeks to wild boar meat infected with *T. britovi*, inactivate the larvae, canceling their infective mice capacity.

Key words: *T. britovi* larvae, wild boar meat, controlled freezing, viability

0084 *Trichinella* infection in Serbia, from 2014 to 2018

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The aim of this work is to present epidemiological and epizootological data on *Trichinella* spp. presence in Serbia for the period 2014 - 2018. During this period about 2.3 million pigs were slaughtered annually and examined in slaughterhouses, veterinary ambulances, stations and Institutes for the presence of *Trichinella* larvae in meat. The rate of domestic swine infection decreased from 0.007% to 0.003% between 2014 and 2018. This represents a constant improvement comparing to previous five years period (for which an average value of 0.018% for the infection rate was observed). The prevalence in wild boars varied from 1.267% in 2014 to 0.556 in 2018. In spite of the fact that the number of above mentioned *Trichinella* positive animals, recognized as a main sources of trichinellosis in Serbia, decreased during years, the number of human cases remained similar until recently. While for the period 2014-2016 there were 358 cases of trichinellosis (22 outbreaks, no lethality), a significant decrease was noticed for 2017 and 2018. There were only 15 cases (2 outbreaks) in 2017 while in 2018 there were no outbreaks but the presence of 11 sporadic cases was registered. The biggest outbreak for 5 years period took place in the Cajetina, Zlatibor District (contributed 111 out of 190 cases reported in 2016). While the presence of the *T. spiralis* in a meat samples predominate as infection source in Serbia, in this outbreak the etiological agent identified in the meat of wild boars was *T. britovi.* In all human cases the source was *Trichinella* containing meat and/or meat products prepared without parasitological investigation. Homemade meat products intended for personal usage had often been distributed among relatives and friends in Serbia and abroad, representing a highly appreciated but dangerous gift if prepared from untested and infected meat. During here presented 5 years period there were one exported (France, 2017) and one imported (Bosnia and Herzegovina, 2017) outbreak of trichinellosis. Decrease in annually repeating outbreaks of trichinellosis indicates increased awareness of the risk of the disease and could be the consequence of the enforcement of the measures introduced in education and prevention. (Acknowledgement: Project No. 173047, Ministry for Education, Science and Technological Development, R. Serbia).

0065 The impact of the climate change and human behavior on the biology and epidemiology of *Trichinella*

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The interaction between the environment and the *Trichinella* biology is much stronger than it might assumed for these endoparasites, which apparently do not have a free-living stage. It follows that the climate change could have direct effects on the natural cycle of *Trichinella* spp. by increasing/decreasing the survival of the larval stage in carcasses, and indirect effects affecting the biology of *Trichinella* hosts. Increased humidity favors the survival of larvae in muscles of host carrions, whereas increased drought has the opposite effect. Increased temperature accelerates the decomposition of host carcasses, thus reducing the survival time of larvae in their decaying muscles. Reduction of snow cover limits the survival of muscle larvae in host carcasses. Increasingly frequent extreme weather events may cause the death of wild animals whose carcasses can be transported by water to long distances favoring the spread of *Trichinella* spp. Ozone depletion could reduce the survival and infectivity of larvae in carrions. Humans have caused a radical change in animal biomasses with a strong reduction of carnivores and a high increase of domestic and wild swine. The introduction of alien host species, which act as reservoirs for *Trichinella* spp., may increase the parasite biomass. In the last century, 44 alien mammalian species reached Europe, including carnivores such as the American mink, raccoon, raccoon dog, and jackal, all excellent *Trichinella* spp. reservoirs. Changes in animal behavior such as the urban fox phenomenon should be monitored due to the changing feeding behavior of foxes. Industrial livestock are generally free from foodborne zoonotic parasites. Illegal importation of meat can be the source of human outbreaks when introduced by personal baggage such as wild boar and pig meat infected by *Trichinella*, introduced from Eastern to Western European countries. From 1975 to 2005, 15 trichinellosis outbreaks were documented in France and Italy due to the consumption of horsemeat imported from

Eastern European countries or from North America, highlighting the relationship between meat trade, globalization and eating habits. Hunters who leave animal carcasses in the field after skinning, or remove and discard the entrails, or dispose of in dumps favor an increase of *Trichinella* spp. biomass in the environment, as is the case of *Trichinella* susceptible animals killed by cars whose carcasses are left behind at the roadside. To overcome these problems, there is the need to train and educate farmers, hunters, consumers, public administrators and politic makers on this zoonosis.

0066 Trichinellosis scenarios in people, domestic and wild animals in South America

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In America, such as in other continents, *Trichinella* infection is a health problem for humans and a negative impact for the pork meat market, generated by the apprehensiveness of people to become infected with the parasite. In South America, human infection with *Trichinella* has been documented in Argentina, Bolivia, Chile and Ecuador. Published data of samples of pigs indicated that 13294 from Brazil, 1967 from Colombia and 185 from Peru were negative for artificial digestion or ELISA tests. During the period 2012/2018, in Argentina, suspected cases of human trichinellosis were 6690, however only 2386 were confirmed. The provinces more affected were: Buenos Aires, Santa Fe, Cordoba and La Pampa, also, human cases were

detected in southern and eastern provinces, where the infection has been historically rare, such as Mendoza and Corrientes. In Chile, 258 human cases of trichinellosis were confirmed in the period 2005/2015, where the largest number of positive samples for *Trichinella* spp (29.5%) was detected in the Metropolitan district (center of the country) and 17.4% in The Lakes district (southern Chile), while the most affected were people between 30 - 49 years of age (40.1%). There was an increase of human cases during autumn and winter in Argentina due to the consumption of raw pork and wild boar preparations, and in winter and spring in Chile, which was associated to the Mapuche New Year and National Holidays Celebrations. The serological prevalence in Bolivia was 3% in humans and 13.4% in swine, while in Ecuador 5.72% in free roaming pigs. Four *Trichinella* species were identified in South America: *T. spiralis*, *T. patagoniensis*, *T. pseudospiralis* and *T. britovi*. *T. spiralis* is the most prevalent species. *T. patagoniensis* was found in natural conditions in cougars from Argentina, but nevertheless under experimental conditions, was able to infect the muscles of cats and guinea pigs. *T. spiralis* was identified in a South American sea lion (*Otaria flavescens*) from Patagonia, Argentina, for the first time in the region. With regard to animal species used as food in the continent, the most infected were pigs, wild boars and cougars, while armadillo and peccary showed very low level (0.04- 0.1) of larvae per gram (lpg). Considering the increase of the guinea pig meat market in America and wild boar from hunting, which add to the pork market or other wild animals it is necessary to improve regulations and diagnosis in meat before consumption, to avoid the transmission of this zoonoses to people.

0073 *Trichinella* spp. in susceptible wildlife from swine-producing regions of Canada

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The OIE (World Organization for Animal Health) Terrestrial Animal Health Code stipulates requirements for establishing and maintaining compartments of domestic swine under controlled management conditions to ensure negligible risk for *Trichinella*. This includes knowledge of *Trichinella* spp. in susceptible wildlife, particularly regarding those genotypes of the parasite known to be infective to swine. Most surveillance of Canadian wildlife for *Trichinella* spp. has entailed the opportunistic testing of samples from the far north and other remote regions where domestic livestock are absent. A survey was therefore initiated 5 years ago to specifically target wildlife from agricultural regions conducive to swine production across the country. To date, over 1400 samples have been tested from muscle predilection sites of 20 species of omnivorous or carnivorous wildlife, including rats, from six provinces where the vast majority of pigs are produced (British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, and Quebec). Samples were tested using artificial digestion (double separatory funnel method), and recovered larvae genotyped by multiplex PCR. As expected, the most frequently identified genotypes have been *T. nativa* and *Trichinella*-T6, but findings of *T. murrelli* in southern Ontario and *Trichinella*-T6 in Saskatchewan represent new host/geographical occurrences of these species. None of the results obtained thus far from this ongoing survey indicate a significant risk posed by *Trichinella* spp. in wildlife to Canadian swine.

0053 Epidemiology and new trends in wild boar trichinellosis in the Czech Republic

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Trichinellosis is a food borne zoonotic disease caused by the consumption of raw meat and raw meat-derived products from animals infected with nematode larvae of the genus *Trichinella*. In Europe, the parasite is more prevalent in wildlife than in farmed animals and wildlife animals serve as the major reservoir hosts. The aim of the present work was to evaluate the overall prevalence of *Trichinella* spp. infections in wild boar hunted in Czech Republic over a 18 year interval (from 2001 to 2018). Between years 2001 and 2018, more than 2,2 million wild boars (*Sus scrofa*) were hunted and of these 1,806 million (80,85%) were tested for *Trichinella* sp. in the Czech Republic. *Trichinella* infection was demonstrated in 27 wild boars (prevalence 0,0015%). Although the prevalence of *Trichinella* spp. infection in wild boars is very low, the spatial analysis reveals that the level of risk differs by region in the Czech Republic. Larvae of 27 *Trichinella* isolates were identified as *T. britovi* (15; 55,85%), *T. spiralis* (8; 29,63%) and *T. pseudospiralis* (4; 14,82%); no mixed infection was not found. Two new trends of wild boar trichinellosis were observed during the last five years. Firstly, the number of hunted wild boars tested in the Czech Republic has increased and reached almost 100% of hunted wild boars in 2015 and 2016. Secondly, two cases of trichinellosis were observed in wild boars imported to the Czech Republic. Larvae of *T. britovi* were found in wild boar imported from Podkarpackie Voivodship in Poland and three *T. spiralis* positive wild boars were detected in group of 182 imported animals from Hajdú-Bihar County in Hungary. Both new aspects will be discussed in context of EU legislation during presentation.

Keywords: trichinellosis, wild boar, prevalence

0060 Isolation and Species Identification of *Trichinella* sp. from Farmed Minks *(Neovison vison)* in Shandong Province, China

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Trichinellosis is an important parasitic zoonosis caused by the nematode genus *Trichinella* with a worldwide distribution. The parasite can infect a broad spectrum of mammal, especially the carnivore and omnivore animals that can act as the reservoir hosts. However, little is known of *Trichinella* infection in Minks (*Neovison vison*) from Shandong Province, China. The objectives of the investigation were to examine *Trichinella* prevalence by artificial digestion in farmed Minks from Shandong Province, China and identify the species through PCR amplification of the expansion segment V region of the ribosomal DNA (5S rDNA). For each Mink, 5 g of muscle tissues were collected, and a total of 50 g of muscles was pooled for the artificial digestion. Nearly 200 larvae were isolated from 20 Minks. The parasite was then reserved in Kunming mice. Analysis of the 5S rDNA inter-gene spacer region from the isolates revealed that it is identical to the corresponding sequence of *T. spiralis* isolate Ts1 from GenBank (Sequence ID: MH535980.1). To the best of our knowledge, the present study represents the first report of *T. spiralis* infection in Minks from Shandong province, China.

Keywords: *Trichinella spiralis,* Mink, China, Artificial digestion, Species identification

0057 The raccoon dog as reservoir and vector for *Trichinella* in Germany

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Due to their distinct scavenging behavior, raccoon dogs can play a significant role as *Trichinella* reservoir. The raccoon dog population in Germany has risen considerably over the past years. From only 58 raccoon dogs in 1987, the nation-wide hunting bag 15 years later was approximately 12,000 raccoon dogs, with 96% of all animals shot in the North-Eastern part of the country.

The *Trichinella* prevalence in outdoor domestic pigs and the wild boar population in North-Eastern Germany are significantly higher in comparison to the rest of the country. This study is an ongoing effort to determine the role of the raccoon dog as reservoir and vector for *Trichinella* in Germany.

The migratory pattern and migration speed of the raccoon dog in Germany was determined based on the size of the hunting bag. Further, a total of 1648 animals from the Eastern federal state Brandenburg were examined for *Trichinella* spp. according to Regulation (EC) No. 2015/1375. The parasite burden was calculated as larvae per g muscle weight and the *Trichinella* species was identified by multiplex-PCR.

The data show that between 2008 and 2017 the nation-wide hunting bag increased by 60% despite a canine distemper outbreak in 2010/11 and has not yet reached a plateau phase. Sustainable raccoon populations from North-Eastern Germany have spread in westerly and southerly direction at a median speed of 8.6 km.

Two percent of the examined animals were infected with *Trichinella* larvae. More than 90% of all isolated larvae were typed as *T. spiralis*. The parasite burden in musculature ranged between 0.5-744 larvae per g. The other three isolates were identified as *T. pseudospiralis* (3.9 larvae per g) and *T. britovi* (36 and 210 larvae per g).

Findings of this cross-sectional study show that the 2% *Trichinella* prevalence in the raccoon dog population in Brandenburg is significantly higher compared to the average prevalence rate found in wild boars (< 0.004%) and foxes (< 0.5%). The reservoir competence of the raccoon is emphasized by the high larval muscle burdens found for both *T. spiralis* and *T. britov*i. The question remains, if the spread of the raccoon dog in Germany will result in an increase of the *Trichinella* prevalence in the sylvatic cycle. The data highlights that the carcasses of raccoon dogs and other wild animal species susceptible to *Trichinella* infection should be appropriately disposed of to avoid the spread of this zoonotic parasite.

POSTER PRESENTATIONS

0034 *Trichinella* spp. findings in Austrian wildlife between 2011 - 2018

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Trichinella spp. is the causative agent of human trichinellosis and circulates predominantly within the sylvatic cycle in Austria. Red fox (*Vulpes vulpes*) act as main reservoir of infection. No positive findings have been reported in Austrian fattening and breeding pigs for over decades.

In wild boars (*Sus scrofa*), the yearly hunting bag ranges between 40,000 and 50,000 animals with the number increasing. Wild boars undergoes *Trichinella* examination by either artificial digestion method or trichinoscopy according to the Regulation (EC) No. 2015/1375. Before 2011 findings of *Trichinella* in wild boars were very rare and not well documented. As molecular diagnosis for species differentiation was not established during that time, the actual *Trichinella* species was unknown and all larvae were identified as *Trichinella spiralis*.

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

Within a surveillance and monitoring program between December 2013 and February 2018, muscle tissue of 1379 red foxes, originating from Alpine areas of the provinces Salzburg, Tyrol and Vorarlberg were collected and examined by artificial digestion. The samples were taken from the front leg and 10 gram were digested. In 42 foxes larvae were identified as *Trichinella* spp. based on morphology. This means an overall prevalence in all examined samples of about 3.04%. Out of the 42 individuals 16 were female and 26 were male. The infected foxes harbored larvae in the range of 0.2 to

65.4 LPG with an overall of 8.72 LPG. All positive foxes were infected by *Trichinella britovi.*

Additionally to the mentioned wildlife species a case of *Trichinella* infection was confirmed in a badger (*Meles meles*). The larvae were identified as *Trichinella britovi*.

0024 Croatia: Trichinellosis outbreak due to consumption of homemade smoked wild boar meat products

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Trichinellosis is a serious and sometimes deadly disease caused by a parasite from the *Trichinella* genus. In over 60% of the reported epidemics, it is the cause of the consumption of thermally insufficiently processed pork infected with the parasites from the *Trichinella* genus. While the risk of developing diseases due to the consumption of domestic pig meat in many countries has been reduced or completely eliminated due to modern production and control of meat, wild boar meat for specific reasons still poses a risk.

In Croatia trichinellosis was a disease of public importance during the last decade of the last century. However, the epidemiological situation has been significantly improved to date. Due to the institutionalized approach in addressing each epidemic, we have noted new trends in the epidemiology of trichinellosis in the last couple of years.

The paper presents the epidemic of trichinellosis in a town in the eastern part of Croatia, which is considered an endemic area of trichinellosis. The epidemic was registered at the beginning of 2017 when a young married

couple was taken to a hospital because of stomach cramps, diarrhoea, one-time vomiting, fever up to 38° C, myalgia in hands and feet, conjunctive redness and eosinophilia in the male member, and somewhat milder symptoms in the woman. Anamnesis stated that domestic cured meat products were consumed on multiple occasions. A total of 69 individuals were exposed to the infection, of whom 26 were diagnosed with trichinellosis (clinical trials, eosinophilia, paired sera); two were hospitalized, 20 were treated as outpatients, and four were asymptomatic (positive serology and eosinophilia in the blood). The patients were treated with mebendazole 3x200 mg for 14 days, and others were recommended mebendazole 3x200 mg for 5 days.

After the examination in the NRL had confirmed that the products were positive to *Trichinella* infection, further analysis showed that the level of the infection ranged from 1.65 to 7.08 L/g. Molecular examinations (Multiplex PCR) confirmed *T. spiralis* in all samples. Due to the extremely unreliable information on the origin and type of meat used for the preparation of meat products, we opted for the real-time PCR and the objective evidence of the meat origin. The presence of domestic pig (*Sus scrofa domesticus*) and wild game meat (*Cervus elaphus* and *Capreolus capreolus*) was excluded and the presence of wild boar meat (*Sus scrofa srofa*) was confirmed.

0014 New host record for *Trichinella britovi* in Romania: the European badger, *Meles meles*

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The European badger, *Meles meles* (Carnivora, Mustelidae), is an opportunistic omnivore that can be found throughout Romania, with an estimated population of over 20000 individuals in the country. They feed on a wide variety of plants and small mammals, occasionally preying on rodents, such as mice or rats. Considering that rodents are known to play a key role as reservoirs in the life cycle of *Trichinella* spp., the aims of this study were to investigate the occurrence of *Trichinella* spp. in badgers from Romania and the identification of the parasite species. Overall, 61 badger carcasses originating from 14 counties were examined by trichinoscopy and artificial digestion. For species determination, the positive muscle samples and the larvae recovered from artificial digestion were submitted for DNA isolation and further processed by means of Multiplex PCR. A single badger, originating from Sibiu County, Central Romania, was positive for *Trichinella* spp. Five larvae were identified during trichinoscopy: four in the diaphragm and one in the foreleg muscles. Artificial digestion revealed an infestation rate of 70 larvae/100 g of muscle. The PCR indicated the occurrence of *Trichinella britovi*, which is the most commonly detected species in wild carnivores in temperate areas. Although *T. britovi* has previously been reported in sylvatic fauna in Romania,, this represents the first report of its occurrence in the European badger in Romania, indicating that badger may be playing a key role in the sylvatic cycle of *T. britovi* in Romania.

Keywords: European badger, trichinoscopy, artificial digestion, PCR, *Trichinella britovi*

0021 The raccoon dog (*Nyctereutes procyonoides*) as a reservoir of *Trichinella britovi* in Poland

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The raccoon dog (*Nyctereutes procyonoides*) is an omnivore originally native to East Asia, and nowadays it is an invasive species in Europe. In 2017, this animal has been included to the List of Invasive Alien Species of Union and it is a concern which poses a significant threat to biodiversity in Europe. It is well known that raccoon dog population is still growing throughout Europe. Literature data show that raccoon dogs act as a reservoir of many parasites of public health importance and possibly this species has a major contribution to the transmission of parasites to other wildlife animals. The aims of the study were to determine the prevalence and muscle distribution of *Trichinell*a spp. in wild raccoon dogs in Poland. Carcasses of raccoon dogs used in the study were collected within the Project Life +, no. LIFE11 NAT/PL/428. Presented research was performed within the National Science Centre, Poland, project no. 2017/25/N/NZ7/02625. *Trichinella* larvae were detected in 45 of the 113 examined raccoon dogs, giving prevalence 44.90% among females and 35.94% among males. The larvae were identified at species level by multiplex polymerase chain reaction (PCR) as described by Zarlenga et al. (1999), with some modifications. All of the isolated larvae were classified as *T. britovi*. The intensity of infection ranged from 0.02 to 622.92 larvae per gram (LPG) (mean 56.06; median 21.44). The difference between the number of infected males and females were statistically significant ($p<0.05$, Chi-Square test) with more females harbouring infection. The highest LPG was recorded in the tongue, lower forelimb and masseter among examined females; and in the tongue, lower forelimb and lower

hind limb among males. Additionally, to examine the intensity of infection, expressed as average of LPG per animal, the results were divided into four groups as follows: <1 LPG, 1-10 LPG, >10-100 LPG and >100 LPG. Our results show that the muscle distribution of *Trichinella* was random in animals with low intensity of infection (<1 LPG group). The highest larval burdens were observed in lower forelimb muscles in the 1-10 LPG and >10-100 LPG groups, in both sexes. Interestingly, in the >100 LPG group, the predilection muscles for *T. britovi* were the masseter in females and the tongue in males. The results of the study show that raccoon dogs act as a reservoir of *T. britovi*, due to the high prevalence of *Trichinella* larvae observed and this may be playing an important in maintaining the sylvatic cycle of this of *T. britovi* in wild environment.

0052 Occurrence of *Trichinella* spp. in carnivore community in Poland

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Nematodes of the genus *Trichinella* are zoonotic parasites maintained by a wild cycle involving mainly carnivorous mammals. In Polish wildlife the presence of *Trichinella* spp. has been confirmed in several mammal species mostly as descriptions of individual cases. There are lacking comprehensive studies analyzing the spread and extent of *Trichinella* infection in species and mammal communities.

In this study we analysed *Trichinella* prevalence and intensity in carnivore community in Poland between 2008 and 2018. The material included 1369 animals belonging to 10 species: American mink (*Neovison*

vison) (N=812), raccoon dog (*Nyctereutes procyonoides*) (N=257), badger (*Meles meles*) (N=104), pine marten (*Martes martes*) (N=74), stone marten (*Martes foina*) (N=68), polecat (*Mustela putorius*) (N=17), red fox (*Vulpes vulpes*) (N=11), Eurasian lynx (*Lynx lynx*) (N=11), wolf (*Canis lupus*) (N=9), and river otter (*Lutra lutra*) (N=6). Muscle samples were examined for the presence of *Trichinella* muscle larvae using standard artificial HCl-pepsin digestion method. Obtained larvae were counted and the numbers of larvae per one gram of muscle tissue (LPG) were calculated. PCR for *Trichinella* species identification were performed.

In total 10.6% of studied carnivores were infected with *Trichinella* spp. (145 out of 1369 ind.). The parasite was the most prevalent in wolf (66.7%), then in red fox (36.4%), raccoon dog (28%), lynx (27.3%), pine marten (20.3%), polecat (17.7%), stone marten (11.8%), badger (5.8%), and Am. mink (3.3%). No *Trichinella* larvae were found in river otter. Higher prevalence in large carnivores and typical scavengers (red fox, raccoon dog) may result from increased consumption of food being a source of *Trichinella*. The highest average infection intensity was detected in two species: raccoon dog – 20.7 (range 0.1-200) LPG and Am. mink – 13.6 (range 0.1-274) LPG. Three *Trichinella* species: *Trichinella britovi*, *T. spiralis* and *T. pseudospiralis* were genetically confirmed among studied carnivores. There were no significant differences in sex bias in *Trichinella* infection in studied species.

Our results indicate that complex carnivore mammal community is involved in the spread of *Trichinella* spp. in Polish wildlife. The interspecies differences in the parasite prevalence are probably connected with different feeding habits of particular species. Our results are the first to show relatively high prevalence of *Trichinella* parasite in Poland which indicates the need to monitor infection in parallel in many wild species, and not – as usual – only in selected hosts, e.g. wild boar or/and red fox.

0087 "Know to prevent" in Northern Patagonia, Argentina

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Trichinellosis is endemic in Argentina and an important public health problem because of its high morbidity rates. In Patagonia Argentina, despite not being an important pig production area, there are outbreaks of trichinellosis due to the consumption without bromatological control of pigs meat produced domestically and wild animals meat obtained by hunting activity. Also, the production of sausages ("chacinados") for family consumption and informal sale contributes to the transmission. Even though one way to prevent trichinellosis is to cook meat to safe temperatures, the consumption of raw sausages should only be made after the artificial digestion method was performed on animal carcasses and a negative result was given. In this context, the aim of this study was to make workshops for children between 6 and 12 years old, tending to provide tools that reduce the appearance of outbreaks of trichinellosis in a rural area of the northern Patagonia Argentina. Under the concept of "know to prevent", the different aspects of the parasite-host-environment triad were worked on. Between August 2016 and December 2017, seven groups of students between 6 and 12 years old of two primary rural schools took part in workshops. To stimulate learning, parasites were observed through a microscope and magnifying glass. They were developed theoretical content and practical, creative and playful activities. In addition, attractive and easy-to-understand brochures were designed for children and families. As a result, 176 children from two rural primary schools in a rural area of the northern Patagonia Argentina learned for the first time what trichinellosis is, how it is transmitted and how it is prevented. To promote a necessary change of attitude tending to prevent trichinellosis, the active participation of children is essential.

0093 Detection of *Trichinella* spp. in Farmed Wild Boars *(Sus scrofa)* in Jilin Province, Northeast China

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The zoonotic trichinellosis is a parasitic disease of public health significance, caused by infection with larvae of the genus *Trichinella*. Pig and wild boar meat are considered the second important source of outbreaks of human trichinellosis, as reported in several countries. However, no reports are available about *Trichinella* infections in wild boars (*Sus scrofa*) in China. The aim of the current study was to investigate the presence of *Trichinella* infections in farmed wild boars (*Sus scrofa*) in Jilin province, northeastern China and its potential risk to humans. In an 11-month survey, a total of 882 serum samples were obtained from farmed wild boars from three cities (Jilin City, Siping City, and Baishan City) in Jilin province, Northeast China. They were tested for antibodies specific for *Trichinella* spp.. using ELISA as described in the OIE guidelines. The prevalence of *Trichinella* infection in wild boar samples was 2.61% (23 out of 882). The highest seroprevalence was observed in animals from Jilin city (3.56%, 10/281) followed by Fusong (2.96%, 10/338) and Siping (1.14%, 3/263), but the difference was not significant. A slightly higher seroprevalence was detected in female animals (2.66%, 20/751) than in males (2.29, 3/131) with no significant difference.

The muscles from *Trichinella* seropositive samples were were screened for *Trichinella* larvae by artificial digestion according to the OIE description. Larvae from positive animals were identified to species level using by PCR-based methods using mitochondrial small subunit ribosomal DNA (mt SSU rDNA) and expansion segment V (ESV) region of the ribosomal DNA repeat sequences as genetic markers. The isolates showed identical DNA banding pattern compatible with *Trichinella spiralis.* To further examine the potential risk of wild boars for human infection, a total of 975 human serum samples were collected from the First Hospital of Jilin University and screened for *Trichinella* seroprevalence by a commercial colloidal gold test kit. Results were negative. These findings shows that *Trichinella* infection was prevalent in farmed wild boars, which is of public health concern. Integrated strategies and measures to control *Trichinella* infection are necessary in farmed wild boars in China.

Keywords: *Trichinella*, Wild Boars, China, Seroprevalence, Artificial digestion, Species identification

0068 *Trichinella* in wild boar: analysis of long-term serological surveillance in Poland

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Serological examination are widely recognized as suitable for monitor *Trichinella* spp. at population level, since digestive method it is labor intensive and less sensitive than serological techniques. The aim of the study was to determine the threat of trichinellosis in different part of Poland. As a tool for

monitoring, the QIAGEN Trichinella Ab ELISA test was chosen, test was formerly validated by the F. Loeffler Institute in Germany. Material for the study were wildboar serum samples collected by official and designated veterinarians in 2014 – 2018. In total 7776 wildboar serum samples were collected. Examination was were performed in accordance with the methodology given by the test manufacturer. Optical density measurement was read on the MRX 2000 spectrophotometer at 450 nm. Positive results were obtained in 874 samples, which is 11.24%.The highest seroprevalence over 20% was observed in 2 out of 16 regions (Łodzkie and Wielkopolskie region respectively 21,3 and 20.38%) with median 9,7%. In recent years, there has been a change in the characteristics of trichinella occurrence in wildboar population in the north-western part of the country. Until now, trichinellosis of wildboars appeared sporadically. The observation of trichinosis in the Koszalin poviat indicates the change in the nature of infection from disseminated to focused one. In 2016 in Manowo in wildboar population new concentrated type of *Trichinella* infection occurred. New type is characterized by the presence of *Trichinella* spp. larvae in an unique number of animals. The percentage of wild boars infected in one hunt reached 90% (17 out of 19) the average number of infected wild boars in the Manowo area (120 km2) reached 70% of hunted wild boars. The retrospective analysis indicates 5-fold increase in the number of infected animals during last 7 years (from 11 to 56 animals). Sequence analysis of molecular markers indicates homogeneity of *T. spiralis* collected from the animals in this region. It is necessary to recognize the mechanism of the formation of such clusters of trichinosis in the wild animal population. If the mechanism of such clustering is not clarified, it will not be possible to prevent the increase in of the disease in environment. Natural balance has been destabilized and is no longer subjected to self-regulatory processes. This condition requires immediate action to reduce the number of infected animals. Without taking such measures as introducing restrictions related to the collection of carcasses and the utilization of unfit for human consumption parts of animals, systematic risk control, agricultural education, we will be the witness of more frequent cases of trichinosis.

0097 *Trichinella spiralis* natural infection in *Otaria flasvecens* from Patagonia, Argentina

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In Argentina trichinellosis is an endemic disease representing an important risk for human health due to its high rates of morbidity, mainly transmitted by the consumption of raw or undercooked pork. Nevertheless, the discovery of new *Trichinella* species have led to a change in the study of the epidemiology of the disease with the addition of new sources of infection. Moreover, *Trichinella* infection has been detected in a wide range of marine mammals around the world. Until the present time, *Trichinella* spp. infection has not been detected in marine mammals of South America. Four South American sea lions were found dead in the rookeries of Caleta de los Loros (Lat. 41° 00′ S; 64° 12′ W; *n* = 1), Promontorio Belén (Lat. 41° 09′ S; Long. 63° 48′ O; *n* = 1) and Punta Bermeja (Lat. 41° 09′ S; Long. 63° 09′ O; *n* = 2) in Rio Negro, Argentina. Muscle samples were taken from the tongue and diaphragm and were stored at 4 °C until examination at the Parasitology Laboratory of the Facultad de Ciencias Veterinarias, Universidad de Buenos Aires. The total muscle samples from each animal were analyzed by artificial digestión. Identification at the species level was made by nested multiplex polymerase

chain reaction (nested multiplex PCR) based on nuclear ribosomal DNA sequences, using six pair of primers. *Trichinella* spp. larvae were found in one of the four South American sea lions. Based on their morphology, the recovered larvae were suggestive of *Trichinella* spp*. Trichinella* larvae generated a fragment of 173 bp corresponding to *T. spiralis* expansion segment V (ESV) region of the ribosomal DNA.

This is the first report of a *Trichinella* species infecting marine mammals from South America. The inclusion of *Otaria flavescens* in the wide range of *Trichinella* hosts adds new questions to the epidemiology of *Trichinella* in marine animals.

We thank the Secretaría de Medio Ambiente y Desarrollo Sustentable (SAyDS) of Río Negro Province for giving us permission to get sample from the rookeries.

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0108 *Trichinella* in wildlife in Sweden 2007 - 2018

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This presentation summarises the results of *Trichinella* testing in Swedish wildlife during 2007-2018.

The Swedish wild boar population is steadily expanding in numbers and localization. Thus, since 2007 the annual hunting bag has increased from ca 33 000 to 115 000 animals. The estimated proportion of the hunted boars that were tested for *Trichinella* increased from 50% in 2007 to over 90% in 2017. However, the *Trichinella* prevalence was very low (0.1-0.01‰) without any apparent trends over time. The most prevalent species were *T.*

pseudospiralis (48%; 22/47 positive cases) and *T. britovi* (41%; 19/47), while *T. spiralis* was only found twice (4%) and in three (7%) cases the species was not identified. Also, *T. nativa* was found once as a mixed infection with *T. britovi*.

Hunted brown bears (*Ursus arctos*) that are consumed should be tested for *Trichinella,* while testing of other sylvatic animals is limited to those sent to SVA for general or targeted wildlife disease surveillance. Thus, the animal species most frequently tested were bear, wolf, lynx and red fox (n=2 706, 405, 1 363 and 1 795, respectively). The prevalence ranged from 6-7% in lynxes and wolves to 0.4-0.7% in bears and red foxes. In these four hosts *Trichinella nativa* was the dominating species (67%; 90/135) followed by *T. britovi* (13%; 18/135) (including three cases with both *T. nativa* and *T. britovi)*. A few cases with *T. spiralis* (2%; 3/135) were also detected.

During this 11-year period there were no obvious trends over time in the prevalence in any of the host species. However, the prevalence in red foxes (0.7%; 95% CI 0.3-1,1%) was significantly lower than the 4.5% (95% CI 3.5-5.5%) reported for the period 1985-2003, while the prevalence in the other hosts were similar to those previously reported (Pozio et al., 2004).

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HUMAN TRICHINELLOSIS

ORAL PRESENTATIONS

0018 First description of *Trichinella papuae* involved in an outbreak in central Kampong Thom province in Cambodia

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At the end of September 2017, a severe trichinellosis outbreak occurred in Prey Long (Sandan district), Kampong Thom Province (Cambodia). Following consumption of a raw wild pig (*Sus scrofa*) mid-August in a forest during an exercise, 33 military got infected by the nematode and 8 among them died. Patients were hospitalized in Kampong Thom Province

Hospital and in Phnom Penh (Preah Ket MeaLea and Calmette Hospitals). The medical records of 25 patients were collected and analyzed through clinical symptoms, blood analysis, diagnosis and treatment. The clinical symptoms included myalgia, facial and/or lower extremity oedema, headache, fever, diarrhoea, abdominal pain, nauseas and asthenia. Increased CRP, CPK and AST were noted as well as white blood cells counts and in particular eosinophilia. Histopathological preparation on muscle biopsy was done for 13 patients and revealed the larvae. For 11 other patients, an ELISA has shown the presence of IgM (5/11) and IgG (10/11). Remaining biopsy samples were digested and larvae were retrieved and counted. Parasite DNA was extracted and multiplex PCR and PCR (targeting expansion segment 5, internal transcribed spacer and 5S ribosomal DNA intergenic spacer) followed by sequencing ascribe the parasite to *Trichinella papuae*. This non-encapsulated species was first described in Papua New Guinea in 1999 and several outbreaks were recorded in Thailand in 2006 and 2007. The 25 patients were treated with albendazole (400 mg) during about 15 days and received supportive care (prednisolone for example). This is the first description of *T. papuae* in Cambodia and in a fatal outbreak.

Keywords: *Trichinella papuae*, Cambodia, outbreak, biopsy, serology

0004 Backyard pigs: a common source for a trichinellosis outbreak reported in France and Serbia in 2017

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Backyard-pig meat was a common source for a trichinellosis outbreak which emerged in France and Serbia in 2017. The index cases were exposed in Serbia and brought back to France pork delicatessen which was shared with relatives and friends. Around 40 individuals were exposed to the parasitized meat in France and Serbia and 20 cases of trichinellosis were reported (9 in France and 11 in Serbia). Diagnosis was delayed due to miss-acknowledgment of the parasitosis and led to complications in French cases: facial paralysis and pulmonary embolism.

Keywords: trichinellosis, *Trichinella spiralis*, pork, travel, Europe

0064 Trichinellosis in Italy from 2005-2016: a retrospective study based on the analysis of hospital discharge records

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In Italy, as well as in most of the European countries, the notification of *Trichinella* infections in humans is mandatory, however, no information is available on the number of cases occurring annually and this is mainly due to the absence of pathognomonic signs and symptoms. The aim of the present study was to retrospectively evaluate the burden of trichinellosis in Italy from 2005 to 2016, based on hospital discharge

records (HDRs). Results were then compared with the Italian National Reference Laboratory for *Trichinella* (NRLT) reports, the European Centre for Disease Prevention and Control (ECDC) reports and literature data. During the studied period, 102 HDRs showing the identifying code for trichinellosis (#124) were registered. Their screening revealed that, based on the ECDC case definition, the 124 code was correctly reported in 30 (29.4%) records only. From these records with a correct diagnosis of trichinellosis, nine cases were reported by HDRs only, 21 cases were documented by both HDRs and the NRLT, whereas the NRLT documented 106 additional cases. The trichinellosis average yearly incidence in the studied period resulted to be 0.018 cases per 100,000 inhabitants. Out of a total of 136 cases documented in the investigated period, 54 (39.7%) patients were hospitalized. In this study, information on the etiological agents was available in 98% of cases. *Trichinella britovi* was documented in 56% of infections, *Trichinella pseudospiralis* in 27% and *Trichinella spiralis* in 15%. The main source of infection was meat and meat derived products of illegally hunted wild boar (65%), followed by free-ranging pigs (29%), and horse meat imported from abroad (5%). This study highlighted the limitations of the use of HDRs in obtaining true data on prevalence/incidence of trichinellosis in Italy since only a small percentage of patients with trichinellosis is hospitalized and outpatients are neither reported nor registered by HDRs. On the other hand, the surveillance system used by the NRLT successfully recognized 77.9% of infections caused by *Trichinella* species. This study identifies the need to intensify the surveillance system for trichinellosis through the development of an Italian registry. This could allow the identification of patients with severe infections, as well as pauci-symptomatic patients, and will avoid the need for clinical analyses and unnecessary treatments and thus help reduce the consequent economic burden on the Italian National Health Service.

0026 Enhanced oral bioavailability of albendazole against *Trichinella spiralis* infection by nanostructured lipid carriers

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Albendazole is an efficient nematocidal drug with promising effects against *Trichinella spiralis* infection. Unfortunately, its low bioavailability minimizes its effectiveness against the migrating and encysted phases of *Trichinella* infection. Nanostructured lipid carriers (NLCs) are efficient lipid-based drug delivery systems which can enhance the oral bioavailability of albendazole providing greater chance for tackling the migrating and/or encysted phases of *Trichinella* infection. The purpose of this study was to probe NLC for enhanced efficacy of albendazole against *Trichinella* infection. The drug was loaded in precirol based NLC with oleic acid serving as the liquid component. The prepared NLC had an average size in nanoscale range. The effect of albendazole NLC formulation was investigated in comparison with albendazole suspension in different phases of *T. spiralis* infection; intestinal, migratory and muscle phases. Mice were orally infected with 200 *T. spiralis* encysted larvae/ mouse then divided in each phase into three subgroups; control non-treated, albendazole-treated and albendazole NLC-treated groups. Our results showed greater effect of albendazole NLC formulation over albendazole throughout the infection phases as shown by the reduced adult *T. spiralis* count in the intestine and reduced *T. spiralis* larvae in muscles with statistically significant differences. Moreover, the histopathological examination of the infected muscles showed decreased inflammatory infiltration with degeneration and destruction of the encysted larvae in muscles in the groups of mice treated with albendazole NLC in the migratory and encysted phases. In conclusion, albendazole NLC formulation is promising for enhanced nematocidal efficacy of albendazole against *Trichinella* infection.

POSTER PRESENTATION

0002 *Trichinella spiralis* stayed more than 30 years in human tongue

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Human trichinellosis is a cosmopolitan nematodosis rare in France. The parasite reaches the skeletal striated muscles. Diagnosis is based on fever, edema and myalgia associated with blood eosinophilia, increase of creatinine-phosphate kinase (CPK) level, positive serology and skeletal muscle biopsy. This case reports an unusual observation of *Trichinella* larvae stayed more than 30 years in human tongue.

A French 68-years-old female patient, without medical history, has consulted in odontology for a lesion of the tip of the tongue evolving since one year. The examination revealed a bluish soft lesion suggestive of venous angioma. A biopsy was performed and histological examination showed ovoid formations surrounded by cuticle suggesting a round worm encysted in the striated muscle (Figure 1). A reactive aspect of epithelium and chorionic inflammation were associated. Other forms of calcified larvae were also found in the sample (Figure 2).

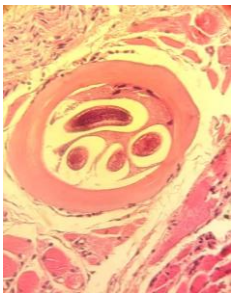

Figure 1

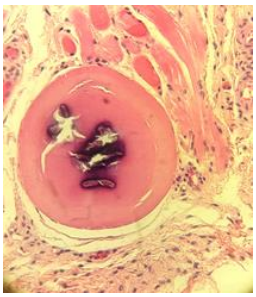

Figure 2

The patient has reported no trip during these last 20 years and has declared being vegetarian since she contracted a trichinellosis 32 years ago. Indeed, in October 1985, a 642 cases outbreak has been reported in south and southern suburbs of Paris causing 3 deaths a few days after having eaten horse meat from Poland. Country of horse slaughtering was Germany. The species involved in this outbreak was *Trichinella spiralis*. This event has made the health surveillance of meat in France mandatory. The patient reported a parasitic treatment by flubendazole in 1985 with rapid decrease of facial and periorbital edema, and myalgia and CPK. On the basis of these new findings, eosinophil blood count and CPK levels have been evaluated in 2017 and were normal. The trichinellosis serology was negative. Specific PCR multiplex *Trichinella* used usually on larvae identified *T*. *spiralis* on tongue biopsy.

This is the first case yielding *Trichinella* larvae in human tongue. This case shows that living larvae can be kept in muscle more than 30 years after the disease and can lead to symptoms.

Keywords: *Trichinella spiralis*, tongue, human trichinellosis

GENOMICS AND PROTEOMICS

ORAL PRESENTATIONS

0089 The roles of serine protease-like protein from the new-born larvae stage of *Trichinella spiralis* in regulating collagen synthesis and differentiation on C2C12 myoblasts in vitro

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Serine proteases have been identified as important molecules that are involved in parasitic infections and host-parasite interactions. In a previous study, a stage specific serine protease was identified by subtractive cDNA library of *Trichinella spiralis* new-born larvae, named *Ts*-NBLsp, which may play a role in installation of the parasite within invaded muscle cells. The aim of our study was to investigate the ability of *Ts*-NBLsp in regulating nurse cell formation using the myoblasts C2C12 *in vitro*. In this study, the full-length *Ts*-NBLsp coding DNA was cloned into the eukaryotic expression plasmid pcDNA3·1(+), and the recombinant pcDNA3·1(+)-*Ts*-NBLsp was transiently transfected into the murine C2C12 cell

line. CCK-8 assay and increased Ki67 mRNA level revealed that *Ts*-NBLsp promoted proliferation of myoblasts. Flow cytometry demonstrated that the transfection with pcDNA3·1(+)-*Ts*-NBLsp plasmid increased the proportion of cells arrested in S phase. The mRNA levels of collagen I, VI and cytokines tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), interleukin-17 (IL-17), and vascular endothelial growth factor (VEGF) were obviously increased in C2C12 myoblasts transfected with *Ts*-NBLsp likewise. Consistent with the increase in mRNA expression, ELISA showed that collagen I, VI and cytokines TGF-β, VEGF levels were also markedly increased in *Ts*-NBLsp transfected myoblasts. In addition, our results showed that the expression levels of muscle-specific proteins desmin, MyHC and MRFs (MyoD1 and myogenin) were reduced in C2C12 cells expressed *Ts*-NBLsp. Collectively, our findings suggest that *Ts*-NBLsp has a role on changing the myogenesis process of skeletal muscle cells and participate in the formation of nurse cells during the muscle phase of *T. spiralis* infection. Research on the biological function of the *Ts*-NBLsp is conducive to providing ideas for the elucidation of the complex mechanisms involved in cell-parasite interactions during *T. spiralis* infection.

Keywords: *Trichinella spiralis*, serine protease, C2C12 myoblasts, collagen

0036 Comparative proteomic analysis of serum from pigs experimentally infected with *Trichinella spiralis*, *Trichinella britovi* and *Trichinella pseudospiralis*

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Recently, proteomics has become a powerful post genomic tool for identifying the characteristic protein pattern in various body fluids of animals suffering from different types of viral, bacterial or parasitic diseases. In case of *Trichinella* and trichnellosis proteomic studies, the vast majority of the research is based on the immunoproteomic approach, where immunoreactive proteins from various stages and different parts or organs of the parasite are subjected to in-depth proteomic analysis. Consequently, most of the available proteomic studies allowed to identify and characterize only *Trichinella* stage-specific proteins reacting with infected host-specific antibodies and did not provide any information about changes in the global proteomic serum profile of the *Trichinella*-infested individuals.

In view of the above, the aim of the present studies was to examine the protein expression profile of serum obtained at 13 and 60 days post-infection (dpi) from three groups of pigs (n=6; each group) experimentally infected with: *Trichinella spiralis* (T1;1000 muscle larvae/pig), *Trichinella britovi* (T3;3000 muscle larvae/pig) and *Trichinella pseudospiralis* (T4; 2000 muscle larvae/pig) compared to uninfected control by two-dimension gel electrophoresis (2-DE) followed by matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectroscopy.

The average±SD intensity of *Trichinella* larvae infection (number of larvae per gram of diaphragm=lpg) was as follows: 89.52±60 lpg for *T. spiralis*, 41.46±20.28 lpg for *T. britovi* and 34.20±32.43 lpg for *T. pseudospiralis*. The comparative proteomic analysis of the T1 group vs control revealed 5 (2 upregulated and 3 downregulated) and 5 (all of them upregulated) differently expressed spots at 13 and 60 dpi, respectively. Experimental infection with *T. britovi* induced significant expression changes of 3 (all of them upregulated) and 6 (5 upregulated and 1 downregulated) protein spots in comparison with the control group at 13 and 60 dpi, respectively. Finally, paired analyses between *T. pseudospiralis* infected group and uninfected control detected 6 (1 upregulated and 5 downregulated) and 2 (1 upregulated and 1 downregulated) differently changed spots at 13 and 60 dpi, respectively. Among these 27 spots, fifteen were successfully identified. Depending on *Trichinella* species triggering the infection and time point of the serum collection, they include: IgM heavy chain constant region, antithrombin III-precursor, immunoglobulin

gamma-chain, clusterin, homeobox protein Mohawk, apolipoprotein E precursor, serum amyloid P-component precursor, Ig lambda chain C region OS, complement C3 isoform X1 and apolipoprotein A-I.

Our results revealed that various *Trichinella* species and different phase of the invasion evoke distinct, characteristic proteomic pattern in serum of experimentally infected pigs.

POSTER PRESENTATIONS

0048 Excretion and secretion product of *Trichinella spiralis* can affect functions of neutrophils

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Neutrophil is a kind of innate immune cells and perform multiple functions when exposed to exotic pathogens to kill them. One of the functions is to release neutrophil extracellular traps (NETs) to capture the pathogens including nematode, such as *Trichinella spiralis* (*T. spiralis*). It is well known that parasites have their own strategies to evade the host's immune response. What strategy does *T. spiralis* take when facing NETs released by neutrophils? In this experiment, we extracted polymorphonucleocytes (PMN) from mouse bone marrow and collected excretion and secretion product (ESP) of adult worms of *T. spiralis*. ESP was then added into the cell culture medium to pre-

treat PMN before stimulated with Phorbol-12-myristate-13-acetate (PMA). It was found that PMN pretreated with ESP could not release NETs after stimulated by PMA for 3h, indicating that ESP can suppress NETs generation. In the process of PMA-induced PMN to produce NETs, neutrophil ROS production and respiratory burst are the key points. Therefore, we examined the effect of ESP on ROS production subsequently, and found that ROS was reduced when PMN was treated by ESP, that is, ESP had potent antioxidative activity. The results of LDH assay indicated that the inhibitory effect of ESP on NETs was not achieved by promoting the death of PMN. Next, we examined the effects of ESP on phagocytosis and cytokines of neutrophils. The results showed that ESP could promote the capacity of PMN to phagocytose bacteria and also had a great effect on the production of cytokines. The expression of pro-inflammatory cytokine IL-1β was decreased, and the anti-inflammatory cytokine IL-10 and tumor necrosis factor TNF-α was significantly increased, indicating that ESP can regulate the immune response of neutrophils. In conclusion, this represents a novel mechanism by which *T. spiralis* can regulate and evade innate immune responses.

Keywords: *Trichinella spiralis*, excretion and secretion product, neutrophils, functions

0039 Comparative analysis of excretory-secretory antigens of *Trichinella spiralis* and *Trichinella britovi* adult worm by two-dimensional gel electrophoresis coupled with immunoblotting

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The detailed knowledge about proteomic profile of different *Trichinella* species is essential for the development of serological diagnostic methods for detecting early-stage infection and for species-specific differentiation as well as for vaccines generation. During intestinal stage of

trichinellosis, the excretory-secretory (E-S) antigens produced by the adult worm (Ad) result in early exposure to immune system and elicit the production of specific anti-*Trichinella* antibodies by the host. Thus, the Ad E-S proteins might provide early diagnostic markers for trichinellosis. However, to the best of our knowledge there has been no report on the serodiagnosis of trichinellosis using *T. britovi* adult worm antigens. The aim of this study was to evaluate the immunological potential of *T. spiralis* and *T. britovi* Ad E-S antigens for the early serodiagnosis. To that end, the purified E-S proteins were analyzed by two dimensional gel electrophoresis (2DE) coupled with protein identification by liquid chromatography-tandem mass spectrometry (LC-MS/MS). To search for immunoreactive proteins that are specifically recognized by host antibodies the Ad E-S proteins were subjected to two-dimensional (2DE)-immunobloting with sera derived from pigs experimentally infected with *T. spiralis* and *T. britovi*. The experiment, conducted in triplicate, were highly reproducible, yielding similar patterns of immunoreactive proteins. Out of 394 protein spots identified in *T. spiralis* proteome 23 were immunoreactive, whereas for *T. britovi* proteome 15 protein spots from total number of 253 were recognized by antibodies presented in the pig sera. In the present study except stage-specific proteins the specific antibodies against *T. spiralis* and *T. britovi* recognized 10 protein spots which were common for both of proteomes.

The current research enabled determination of similarities and differences between Ad E-S proteins of two frequently accuring *Trichinella* species. The characteristic of *T. spiralis* and *T. britovi* profiles is valuable complement of current knowledge about immunoreactive proteins of these parasites. The presented variances between them can be considered as a tool for future differential diagnosis of *T. spiralis* and *T. britovi* infections.

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0091 iTRAQ-based differential proteomic analysis of excretory–secretory proteins of *Trichinella pseudospiralis*

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Trichinella pseudospiralis (*T. pseudospiralis*) is a non-encapsulated intracellular parasitic nematode that can possess strong ability to modulate host immune response. Here, we compared the differentially expressed proteins of ES products in three genotypes of *T. pseudospiralis* ML (from Russia, USA and Australia) using isobaric tags for relative and absolute quantification (iTRAQ)-based technology. A total of 2591 non-redundant proteins were identified, of which 65(146), 72(98) and 43(103) significantly up-regulated (down-regulated) differentially expressed proteins were detected among pair-wise comparisons (RUS vs US, AUS vs US and RUS vs AUS). At the same time, GO annotation, KEGG and STRING analysis were carried out on the screened differentially altered proteins. It was found that

the main biological processes involved included carbohydrate metabolic process, DNA metabolic process, cellular protein modification process and homeostatic process. The majority KEGG pathway were found related to the metabolic pathways, lysosome and protein processing in endoplasmic reticulum. Moreover, All ES proteins expression levels involved in the lysosome pathway were significantly higher in the T4 USA genotype than in the other two genotypes. We also found differences in the expression of some important immunoregulatory proteins between different genotypes of *T. pseudospiralis* ML, such as protein disulfide-isomerase, thioredoxin protein and deoxyribonuclease-2-alpha. Quantitative real-time PCR analysis also confirmed that the changes in gene expression were consistent with those at the proteomic level. This study is the first to quantitatively compare the differential expression of proteins among of the three genotypes of *T. pseudospiralis* ML, and further reveal the possible reasons for the different infectivity and persistence of different genotypes of *T. pseudospiralis* to the host on the level of protein.

Keywords: *Trichinella pseudospiralis*, iTRAQ, Excretory-secretory products, Muscle larvae, Proteomics

LEGISLATION AND CONTROL

ORAL PRESENTATIONS

0049 Survival of *Trichinella spiralis* in cured meat products

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Processing of meat is one possible approach to control meat-borne parasites. Processing methods like freezing, cooking and irradiation are recommended for the control of *Trichinella* in pork, horse or game meats if specific technical conditions are fulfilled. Curing is a widely used preservation process influencing product characteristics such as shelf life, food safety, and taste. As curing methods are characterized by a high parameter variability and predictions about inactivation of parasitic stages in raw meat products are difficult, curing and smoking are not recommended for *Trichinella* control.

The objective of this study was to investigate the survival of *T. spiralis* in cured sausages taking into account salt concentration, a_w, pH, temperature, and time. For this purpose, three different sausage types (short, middle, long ripened) were produced using *T. spiralis* infested pork. The sausages were stored at product specific conditions for up to 36 days. After the different ripening times, sausages were digested using the magnetic stirrer method and the viability of the isolated larvae was assessed using a previously published larval motility test indicating the viability and infectivity of *Trichinella* larvae. Further, pH value and water activity of sausages were monitored over time.

From storage day 7, larvae in sausages without vacuum packaging were no longer viable, whereas the maximum viability time for *T.* spiralis larvae was 32 days in short ripened and vacuum packed sausages. This viability time was about 4 times longer for *T.* spiralis than observed in previous studies in short ripened sausages without packaging. During storage, minor changes of the pH value were noted in middle and long ripened

sausages whereas in short ripened and vacuum packed sausages the pH value remained nearly constant. At the end of the storage, all three sausage types showed similar pH values (between pH 5,3 and pH 5,6). A considerable reduction of the a_w value (from 0,96 to 0,64) was measured in unpacked sausages whereas in vacuum packed sausages the a_w value showed a very slight decrease over time (from 0,96 to 0,95).

Results indicate that a_w value in cured sausages will decrease more slowly under vacuum packaging and thus may significantly prolong the viability of *T. spiralis* larvae.

0094 Current status of implementation of the "One Health" concept in monitoring and control of *Trichinella* spp. infections in Serbia

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In Serbia, trichinellosis is one of the most common zoonotic diseases. For several decades it remained as a very serious problem for public health and animal husbandry. Currently an important achievement in this field is noticed. However, despite the fact that at the national level there was a significant decrease in the infection prevalence in domestic swine population (0.003% in 2018 comparing 0.007% in 2014), 11 sporadic cases of human

trichinellosis in year 2018 was registered, in some districts of Serbia the risk of infection with *Trichinella spiralis* is still high. Moreover, the infection with *T. spiralis* and *T. britovi* in wild life currently presents a greater risk for human infection than before, since the infection among wild boars is spread throughout the country with significant prevalence. In the aim to improve effective control and reduce the risk of *Trichinella* infection, the Veterinary Directorate, as national competent authority for animal health and veterinary public health, in partnership with Ministry of Health and Veterinary Institutes, are making efforts in a further alignment of veterinary legislative with EU requirements and OIE standards. This could not be achieved without effective multi-sectorial collaboration and communication along all relevant disciplines and domains, including national and local authorities, public health and veterinary services and organizations, universities, farmers, food industry, hunters and other stakeholders. The coordinated surveillance system has to be standardized and fully implemented and should include information regarding relevant animal populations (including wild animals), case definition, standard diagnostic procedures, the procedure for case confirmation, notification and reporting of all cases and outbreaks, early warning and rapid response system across relevant authorities. If effectively implemented as multi-sectorial and trans-sectorial approach, the "One Health" concept (focused on *Trichinella* and trichinellosis) can bring the numerous benefits for Serbia. Furthermore, continuous monitoring and identification of still existing gaps could help in overcoming the remaining and/or newly identified problems in animal health, food safety, protection of consumers and environment protection. As strategic, institutional, multi-sectorial surveillance system the "One Health" concept approach, supported by an adequate legal framework for detection, surveillance, prevention, control and reporting trichinellosis and harmonized with EU legislative, remains a priority.

Keywords: *Trichinella,* "One health" concept, risk analysis, multi-sectorial, collaboration

0023 Vaccination with DNase II recombinant protein against *Trichinella spiralis* infection in pigs

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Trichinosis caused by *T. spiralis* is an important public health problem. DNaseII is a well-known acidic endonuclease that catalyses the degradation of DNA into oligonucleotides. Previous laboratory studies have found that the *DNase II* has a high rate of protection against *T. spiralis* infection in mice. In this study, our aim is to further explore the protective effect of *DNase II* against *T. spiralis* infection on Changbai pigs. Forty piglets were divided into blank group, PBS *group*, Freund's adjuvant group and *DNase II* group. Two immunizations were performed at an interval of 4 weeks, each pig was injected 1mg recombinant protein for each vaccination. And then we evaluated the humoral and cellular immune responses to recombinant protein, including the dynamic trend of specific IgG, IgG1, IgG2 and IgM

antibodies levels, as well as the levels of Th1 (IFN-γ, IL-2) and Th2 (IL-10, IL-4) cytokines in serum. Results show that Th1 dominanted Th1/Th2 mix immune response was induced by recombinant protein for all the time or a short period after vaccination. And *DNase II* can induce partial protection against *Trichinella* larvae challenge in pigs, when compared to the control group. The study suggested that DNaseII can be used as a potential candidate of vaccine against *T. spiralis*.

Keywords: *Trichinella spiralis*, pigs, DNaseII, vaccine, protective effect

POSTER PRESENTATIONS

0028 Gamma radiation effect on *Trichinella spiralis* and *Trichinella pseudospiralis* infected wild boar meat

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Irradiation as a method to destroy meat pathogens and to produce secure food for consumption was originally rejected by the consumers. The International Commission on Trichinellosis (ICT) considers an irradiation of 0.3 kGy effective to inactivate *T. spiralis* muscle larvae (L1). The present study aims to find the effect of irradiation in order to inactivate muscle larvae of *T. spiralis* and *T. pseudospiralis* in wild boars. Two animals were inoculated per os with 20000 L1 of *T. spiralis* and *T. pseudospiralis,* respectively. Both animals were euthanized 20 weeks post infection (wpi), 20 g of side ribs, boston butt and shoulder were used to determine the larvae burden and the reproductive capacity index (RCI). Besides, samples of 250 g of these muscles were obtained and vacuum packed for further treatment. The irradiation was undertaken at Atomic Centre of Ezeiza (CNEA – Argentine National Commission of Atomic Energy) and measured with an alanine dosimeter with a minimum and maximum dose of 0.32 – 0.41 kGy, respectively. After treatment, 20 g were cut from the centre of each muscle sample 24 h, 7, 14 and 21 days post-irradiation. All samples were individually processed by artificial digestion. CF1 mice (n=72) were inoculated with 300 L1 from the recovered larvae. Animals were euthanized 42 days pi and each carcass was digested. Moreover, three

mice were inoculated with 600 L1 obtained from the previous irradiated and digested muscle samples in order to recover adult worms. After 72 hours post inoculation, animals were euthanized and the small intestine was removed. The intestine was longitudinally opened and cut in pieces of 5 cm long and placed in falcon tubes with 0.9% NaCl saline solution to incubate for 5/12 h at 37ºC. Prior to the experiment, the RCI for *T. spiralis* and *T. pseudospiralis* was determined as 88.1 and 59.9, respectively. All larvae obtained post-irradiation showed integrity of the cuticle and active motility. No adult worms nor muscle larvae were found of *T. spiralis* or *T. pseudospiralis* in the infected mice with irradiated L1. The present results reinforces the importance of irradiation as a method to inactivate encapsulated and nonencapsulated *Trichinella* species.

The present research was approved by the Committee for the Use and Care of Laboratory Animals (CICUAL) of the Facultad de Ciencias Veterinarias, University of Buenos Aires, under permit number 2018/22. This work was supported by Universidad de Buenos Aires, Secretaría de Ciencia y Técnica Subsidio UBACyT 20020130100336BA and UBACyT 220170200331BA and Ministerio de Ciencia y Tecnología, FONCyT Subsidio PICT-2015-2350 and PICT-2015-3469.

0001 *Trichinella* infection in humans and pigs in Croatia (1995-2014) – results of measures taken

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After the war in Croatia, which lasted between 1991 and 1995, due to socio-economic, political and demographic changes, trichinellosis had spread from then endemic Vukovar-Srijem County to other Croatian counties. With

this research, we wanted to see how the number of pigs infected with *Trichinella* was trending, as well as the number of people with *Trichinella* infections, in the Republic of Croatia and Vukovar-Srijem County during the 20 years after the war, and whether the measures taken against trichinellosis (in form of mandatory meat inspection, rodent extermination, compensation for *Trichinella* positive carcasses, co-financing for *Trichinella* testing of slaughtered pigs, slaughter of pigs in slaughterhouses, removal and payments of all pigs from positive farms, compensation for *Trichinella* positive carcasses, payment of damages to *Trichinella* positive animal owners) were successful. Input data were sourced from Ministry of Agriculture, Institute of Public Health and Croatian Veterinary Institute. The results show that the highest number of pigs with *Trichinella* infection in the Republic of Croatia and in the Vukovar-Srijem County were in 1999 and 2000, but their number began to decline rapidly and continuously. The most infected people were in 1998, after which the number began to decline, with sporadic cases of infection. This shows that the measures taken against trichinellosis have led to significant reduction both in human and animal infections, but must remain in place because we are far from eradicating *Trichinella* from Croatia.

Keywords: *Trichinella* infection, Croatia, Vukovar-Srijem County

ANNIVERSARY CELEBRATION OF ICT

ORAL PRESENTATIONS

0105 Urban Metamorphosis 2.0

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The urban landscape represents the largest scale expression of our desire to live in a created environment apart from the pressures of natural selection. But unlike the uninhabited world around us, cities lack strategies for achieving long-term sustainability. Many cities are toxic environments for its inhabitants, as well as to many uninhabited ecosystems from which we derive essential resources that cities demand. This is due mainly to the absence of a master plan for integrating municipal functions (e.g., mass transportation, equitable resource management, optimizing livability for all age groups) that do no harm to the surrounding landscape. Biomimicry based on the ecological processes governing how temperate hardwood forests behave has the promise of reversing this situation, and at the same time providing a nurturing human habitat that is significantly more nature friendly. Carbon sequestration, water harvesting, in situ food production, renewable energy generation strategies, waste-to-energy management, and efficient, cheap mass transportation systems are the main features of the city of the near future. This approach to managing our own part of the planet has the potential of slowing down or even reversing rapid climate change, allowing all living things on Earth an opportunity to once again evolve at their own biological pace.

0100 A french medical mission in Germany to study the trichinellosis outbreak of Emersleben (1883)

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The first human outbreak of trichinellosis ever identified and reported in France occurred in 1879 (Laboulbène, 1881) and led the medical authorities to fear new episodes of this so far unknown disease. Therefore when the impressive outbreak emerged in Emersleben (German Saxony), including 260 cases and leading 52 to death, the French authorities sent two physicians, Paul Brouardel (1837-1906) and Jacques-Joseph Grancher (1843-1907) to study the disease (Brouardel & Grancher,1884). Their roadmap included to study "*the condition of the emergence of such an outbreak, the potential dangers for the French population and finally, to evaluate if the weak experience of French physicians on the topic could have led to the potential misdiagnosis or ignorance of such an outbreak*". They spent two weeks in Emersleben, collaborating with the local medical authorities and a student of Virchow acted as interpreter. Though arrived two months after the onset of the outbreak, they managed to perform an interesting retrospective survey showing a link between the occurrence of symptoms and lethality and complications. They also confirmed that they had never observed such symptoms in French patients. They met Virchow in Berlin and discussed with him about the risks of importing American pigs. Grancher also performed two autopsies and published precise drawings of larvae entering and transforming muscular fibers. This was in opposition with the theory developed by Chatin in his 1883 authoritative monograph "*La trichine et la trichinose* ". This debate lasted for several years as Railliet (who renamed *Trichina* in *Trichinella*) reports in his 1895 "*Traité de Zoologie Médicale et Agricole* " that: "*according to various authors (Virchow, Leuckart, Grancher, etc.), larvae would localize within the primitive muscular fiber. But J. Chatin, in agreement with G. Colin, Robin etc., showed on the contrary that they were stopping in the interfascicular*

connective tissue and that their penetration into the striated substance, was very rare". The two physicians pursued their brilliant careers. Brouardel was a leading authority in forensic medicine and in public health and hygiene. Grancher was a pediatrician but he had also learned histological techniques and for several years served as director of a pathological anatomy laboratory. Grancher is remembered for his research in the prevention of childhood tuberculosis by isolation but mainly by the fact that, in 1885 as Louis Pasteur was not a physician, he performed the first successful vaccination against rabies on Joseph Meister. This survey improved the knowledge of the disease but no new outbreaks were described until the mid XXth century.

0103 Contributions in control testing and post-harvest interventions for the management of *Trichinella* and trichinellosis

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A principal purpose of the International Commission on Trichinellosis is "the use of sound scientific information to develop and elaborate statements, guidelines and responsible opinions concerning various aspects of the parasite, the disease, and their control for use by national and international institutions and organizations". Throughout the history of ICT, its members have collectively and individually made significant contributions in generating sound scientific data, methods and recommendations for the control of *Trichinella* and trichinellosis, both locally and globally. Simple methods of detection that were developed and implemented many years ago are still commonly used today. Recently, modern technology has provided many advanced tools and a plethora of information that must be carefully interpreted and properly applied for controlling the parasite in the food chain. The ICT has played a lead role in developing recommendations for reliable

testing and pre- and post-harvest control programs, including international guidelines or regulations established by the World Organization for Animal Health (OIE), Food and Agriculture Organization (FAO), World Health Organization (WHO), Codex Alimentarius, and the International Organization for Standardization (ISO). This presentation will provide a general overview of ICT contributions in the area of control testing and post-harvest interventions, and describe specific examples based on my own experience.

0101 Scientific achievements of the last 60 years: From a single to a multispecies concept of the genus *Trichinella*

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The scientific basis that led to the development of a multispecies concept in the *Trichinella* genus originated beginning in 1950s, when scientists began reporting an increasing number of host-specificity peculiarities among different geographic isolates. Several investigators (Z. Kozar, G.S. Nelson, R.L. Rausch) reported that isolates from some wild animals appeared to have poor infectivity in pigs and rats, the major hosts for the domestic cycle, leading to speculation that important geographic variability existed within *Trichinella spiralis* the only species in the genus. Comparative infection results sparked great interest among investigators and led to similar comparative studies with various geographic isolates of the parasite. Two different experimental approaches evolved for characterizing various isolates the comparison of reproductive potentials and the ability of two different isolates to interbreed in laboratory mice. In 1972, thanks to the meticulous method of crossbreeding between male and female larvae and biological characters, the Russian scientists (V.A. Britov, S.N. Boev, B.L. Garkavi) described three new species (*T. nativa*, *T. nelsoni* and *T. pseudospiralis*), breaking the concept that the genus *Trichinella* was monospecific and

widening the host pattern to birds. The description of these species generated an intense debate over their taxonomic validity, however, because of the lack of clear morphological differences among these proposed species and since the concept of sibling species was not yet completely accepted by parasitologists. In 1988, the Nobel Price W.C. Campbell ahead of his time, described four distinct *Trichinella* cycles. The resolution of the taxonomic issues was facilitated by the adoption of new biochemical and molecular techniques for systematics research (A.E. Chambers, J.B. Dame, T. A. Dick, H.A. Flockhart, S. Fukumoto, G. La Rosa, K.D. Murrell, E. Pozio, D.S. Zarlenga). In 1992, the first comparative study, comparing 152 isolates from various host species and geographical regions, identified eight distinct taxa (with the code from T1 to T8), four of which represented the four previously proposed species and one, *T. britovi*, a new species. During the last 27 years, the increasing number of investigations in different geographical regions and hosts coupled with the availability of new and highly sensitive molecular techniques has allowed the description of four new species (*T. murrelli*, *T. papuae*, *T. zimbabwensis* and *T. patagoniensis*) and one new genotype (*Trichinella* T9) along with a more complete phylogenetic, zoogeographical and epidemiological knowledge base (R.B. Gasser, S. Krivokapich, G. La Rosa, E. Pozio, B.M. Rosenthal, D.S. Zarlenga).

POSTER PRESENTATIONS

0056 The International *Trichinella* Reference Centre (ITRC): 30 years (1988-2018) of activity

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The ITRC is the reference laboratory of the International Commission on Trichinellosis (since 1988), of the World Organization for Animal Health (since 1992) and of the European Union Reference Laboratory for Parasites (since 2006). The ITRC was appointed as the repository for *Trichinella* strains and as the source of materials and information for the international scientific community, veterinary and public health services. During 30 years of activity, ITRC supported the scientific community by carrying out diagnostic activity, suppling reference material, organizing proficiency testing, training staff of international institution, developing diagnostic methods for taxon identification, isolate tracing and detection of circulating antibodies, and by collecting epidemiological data. To date, more than 7,500 isolates of human and animal origin from throughout the world were tested and identified at the species and/or genotype level. Information about these isolates (e.g. host species, locality of origin, year of isolation, etc.) were collected in a free access database (https://trichinella.iss.it/). Representative isolates of all currently known *Trichinella* taxa are kept *in vivo* at the ITRC laboratory animal facilities and represent an important source of material for scientific investigations. Serum samples of human or animal origin and muscle juice samples were

analyzed by in-house validated tests (ELISA and Western blot) using the most appropriate specific antigens. Serum samples from *Trichinella*-infected and non-infected pigs were established as international biological standard. Since 2007, the ITRC organizes proficiency testing (PT) on "Detection of *Trichinella* larvae in meat by artificial digestion" and, since 2011, PTs on "Molecular identification of *Trichinella* larvae at species level" for national and international laboratories. More than 250 scientists from more than 80 countries attended the ITRC for training courses on specific diagnostic methods. Scientific collaborations have been and are currently carried out with many institutions and researches all over the world. The ITRC activity has been fundamental to increase the knowledge in the taxonomy, epidemiology, diagnosis and control of *Trichinella* infections, establishing solid bases for the best clinical management and control programs for these zoonotic infections.

DETECTION

ORAL PRESENTATIONS

0035 Relationship between anti-*Trichinella* IgG levels and muscle larvae in long lasting *Trichinella* infections in pigs

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Trichinella spp are still circulating among free-ranging and backyard pigs all over the world with associated human infections. Parasitological and serological investigations showed that, beside pigs with larvae in muscles, there were pigs with detectable levels of anti-*Trichinella* IgG, which tested negative for the presence of larvae in preferential muscles. The aim of the present work was to evaluate in long lasting infections the relationship between specific IgG and presence of infective larvae in muscles of pigs experimentally infected with *Trichinella spiralis, T. britovi* and *T. pseudospiralis*. To this end, twenty specific pathogen-free pigs were infected with 10,000 muscle larvae of each of the three *Trichinella* species. For each animal, blood samples were collected at day zero and every month until the last day of the experiment, in which the infected animals were sacrificed and the preferential muscles digested to determine the

larval recovery rate. Groups of four animals for each *Trichinella* species were sacrified at 60 days post infection (p.i.) and at 6, 12, 18 and 24 months p.i. The anti-*Trichinella* IgG kinetic was evaluated by ELISA using excretory/secretory antigens in serum samples. All infected animals seroconverted at 35 days p.i. but one animal infected with *T. britovi* and three animals infected with *T. pseudospiralis,* which did at 42 days p.i. One year after infection, anti-*Trichinella* IgG were still present in all animals infected with *T. spiralis* or *T. britovi* and in 42% of animals infected with *T. pseudospiralis.* whose sera showed optical density values close to the cut-off. Two years after infection, anti-*Trichinella* IgG were still detectable in all animals infected with *T. spiralis* or *T. britovi*, however optical density values were slightly higher in animals infected with *T. spiralis* than those with *T. britovi*. Sixty days p.i., larvae were recovered from all tested pigs but with a larval burden different among the three species. Six months p.i., no larvae were detected in muscles of *T. pseudospiralis* infected pigs and in 50% of *T. britovi* infected pigs in which the larval burden was very low. One year p.i., no larvae were detected in *T. britovi* infected pigs. Two years p.i., only *T. spiralis* larvae were present in pig muscles. These results show the different biological patterns of the three tested *Trichinella* species in swine and provide useful information to understand the sero-epidemiology of these zoonotic pathogens.

0022 Immunoprevalence of *Trichinella* nematodes in raccoons (*Procyon lotor*) from the Czech Republic, Germany and Poland

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The raccoon (*Procyon lotor*) is an animal native to North America. It was introduced to Europe in the 20th Century, and nowadays it is one of the most widespread non-indigenous species of wildlife. Due to their fast spread and sylvatic lifestyle, raccoons can be a reservoir of many parasites, which could be dangerous to humans and to domestic animals.

There is no literature data concerning on immunoprevalence of *Trichinella* nematodes in raccoons in Europe. The aim of the study was to examine occurrence of anti-*Trichinella* antibodies in meat juice in raccoons.

This study was carried out on 139 raccoons from the Czech Republic, Germany and Poland. To detect the presence of antibodies against *Trichinella* meat juice samples were tested using commercial ELISA kit (ID Screen *Trichinella* Indirect Multi-species, IDvet, France), according to the manufacturer's instructions. The optical density (O.D.) was measured at a wavelength of 450 nm using an EL*800 ELISA automated plate reader (Bio-Tek, USA).

The results of the ELISA testing found seven of the examined 139 meat juice samples to be positive for antibodies to *Trichinella*. Additionally, seven meat juices were considered to be doubtful according to the manufacturer's instructions, however, in one raccoon from Germany, meat juice sample was too little to using it in Western Blot. Therefore, seven positive and six doubtful samples were confirmed by Western Blot method, using specific anti-raccoon antibody (Raccoon IgG-heavy and light chain Antibody, Bethyl Laboratories, Inc., USA). In conclusion, we confirmed the occurrence of anti-*Trichinella* antibodies in 13 of 139 examined animals: nine from Poland, three from Germany and one from the Czech Republic, with the overall prevalence 9.35%.

Our results show that raccoons were exposed to *Trichinella* nematodes in three mentioned countries. The role of raccoons as reservoir, and as possibly contributing to spread of these parasites needs further examinations.

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0054 Low *Trichinella* spp. (Railliet, 1895) Antibodies Detected in Domestic Pigs from Selected Slaughterhouses with Farm Risk Assessment in Bulacan, Philippines

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Trichinella spp. is considered as one of the most widespread food-borne zoonotic pathogen globally. It causes trichinellosis which impacts human public health, swine livestock, and food safety. There is insufficient proof and research on the presence of Trichinella inection in animals in the Philippines. This study aims to update records in the country, by verifying the presence of *Trichinella* spp. from among the most active local swine livestock industry in the country and link its potential presence to animal husbandry practices. For each selected slaughterhouse, blood sera were collected from each pig sample. Blood serum was tested through ELISA for detection of *Trichinella* spp. antibodies. For each sampling site, farm risk assessment was conducted to evaluate potential routes of infection. For this study, a total of 555 blood sera, of which 3 blood sera were detected to be serologically positive (0.54% apparent prevalence with 0.11-1.57 confidence interval). Potential infection routes pointed towards variable feeding of meat and grain waste to the pigs. In summary, the present paper confirms *Trichinella* spp. antibodies detected with very low prevalence in the Philippines and demonstrated the potential utilization of antibody detection in pig blood samples as an efficient and complementary early screening and detection tool

in *Trichinella* detection without sacrificing the pig. These results merit calls for a wider screening and testing for *Trichinella* infection in pigs from other Philippine provinces.

Keywords: *Trichinella* spp., Trichinellosis, livestock, food safety, indirect ELISA

0007 *Trichinella patagoniensis* in wild boars: a first approach

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Trichinella patagoniensis was the latest *Trichinella* species isolated in animals. Therefore the knowledge regarding this new species is limited. According to some studies, *T. patagoniensis* had a different range of host than *T. spiralis*. The present study aims to know whether this species is able to develop on the wild boars, one of the most common hosts of *T. spiralis*. For this reason, 5 wild boars (*Sus scrofa*) were inoculated PO with 20000 *T. patagoniensis* larvae (ISS2311), and 3 animals remained uninfected as control group. Before and post infection (pi) whole blood samples were taken every one week by venepuncture of the jugular vein and collected in tubes with EDTA for eosinophil counts. Three blood smears from all wild boars were done from each day of extraction. After 20 weeks pi, all animals were euthanized. From each wild boar, 9 muscle or groups of muscle samples were taken to determine the larvae distribution. Tongue, masseters, boston butt, oesophagus, diaphragm, intercostal muscles, tenderloin, anterior and

posterior limbs were used. One hundred grams of each muscle were used to determine the larvae distribution. All muscles were freed form fascia and tendons, and digested using artificial digestion. Recovered larvae of each muscle sample were expressed as larvae per gram (lpg). The main infected muscles were tongue and diaphragm; however the larvae burden found was extremely low being the maximum larvae burden found in one muscle 0.08 lpg. Moreover, no larvae were found in intercostal and masseter muscles of any infected wild boar. The eosinophil count began to increase one week pi reaching maximum levels (up to 14%) around week 2 – 4 pi, afterward began to decrease to 1-3% at week 7 pi. The present study shows for the first time that *T. patagoniensis* had low infectivity for wild boars and thus these animals would not represent a risk for the transmission of this parasite in nature.

The present research was approved by the Committee for the Use and Care of Laboratory Animals (CICUAL) of the Facultad de Ciencias Veterinarias, University of Buenos Aires, under permit number 2015/16.

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0005 Evaluation of the "PrioCHECK Trichinella AAD kit" to detect *T. britovi*, *T. spiralis* and *T. pseudospiralis* in muscle tissue of domestic pigs by the automated digestion method Trichomatic-TM35

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The PrioCHECK Trichinella AAD kit (Thermo Fisher Scientific) was evaluated for detection of *Trichinella spiralis, T. britovi* and *T. pseudospiralis* larvae (L) in 35 g pork samples using the automated digestion method

Trichomatic-TM35 (Foss Germany, GmbH/Moritz Geräterepartur UG). Pepsin-HCl digestion using the TM35 instrument was used as standard of comparison. For the AAD kit, an *ad-hoc* adapted TM35 instrument (fast rotation time: 2.5 min; digestion temperature: 60°C) was used, while pepsin-HCl digestion worked on a standard TM35 apparatus (fast rotation time: 3.5 min; digestion temperature: 49°C). In each trial, 72 L of either *T. spiralis* or *T. britovi* were spiked into 9 pork samples (3 groups of 3 samples with 3, 6 or 15 L/sample, respectively). Additionally, 15 samples were inoculated with 0.1g of mouse meat containing an undetermined number of *T. pseudospiralis* larvae. In order to detect potential performance variations among batches, the trial was repeated using 3 different production lots (AAD Kits 1-3) and *T. spiralis*-spiked samples.

The AAD kit-TM35 combination achieved a good digestion performance (undigested material <0.1 g in all cases). All positive (*n*=84) and negative (*n*=8) samples were correctly identified. The total recovery rates of *T. spiralis* using three different AAD kit lots were: Kit 1: 80.5% (58/72 L), Kit 2: 88.9% (64/72 L) and Kit 3: 95.8% (69/72 L). The pepsin-HCl digestion recovered 91.7% (66/72 L) of the spiked larvae. For *T. britovi,* the recovery rates by digestion with the AAD kit (Kit 2) and pepsin-HCl were 84.7% (61/72 larvae) and 94.4% (68/72 larvae), respectively. By analysis of 15 samples spiked with *T. pseudospiralis,* a total of 1,149 (mean 76.6/sample; range 8-264) and 1,253 larvae (mean 83.5/sample; range 22-174) were detected by digestion with the AAD kit (Kit 1) and pepsin-HCl, respectively.

The qualitative results using the AAD kit or pepsin-HCl were identical, independent of the *Trichinella* species and/or kit lot. The quantitative differences were not significant. Therefore, the requirements as an alternative method for the detection of *Trichinella* spp. in pig meat seem to be fulfilled. However, the morphological structure of the recovered larvae appeared to be more frequently affected after digestion with the AAD kit (especially for *T. pseudospiralis*). Thus, in some individual larvae, the visualization of the internal structure characteristics for the genus *Trichinella* such as the stichosome was not possible.

A field trial involving at least three local laboratories experienced in detection of *Trichinella* in meat (including the analysis of ~40 *Trichinella*-spiked samples/laboratory) will be performed before an authorization of this alternative diagnostic method in Switzerland would be granted.

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0090 Characterization of antigenic properties of a cystatin-like protein of *Trichinella spiralis* at its early invasion stage

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The excretory-secretory (ES) antigens from *Trichinella spiralis* muscle larvae(ML)are the most commonly used diagnostic antigens for trichinellosis, there is an obvious window period between *Trichinella* infection and antibody positivity. Intestinal infective larvae(IIL) are first exposed to the immune system of the host, and antigens from the worms may be the earliest marker in the diagnosis of trichinellosis. The high-frequency gene encoding a strongly antigenic cystatin-like protein(*Ts*-CLP) was selected from cDNA library of IIL. The aim of this study was to evaluate the antigenic properties of *Ts*-CLP for early diagnosis of trichinellosis. The histidine-tagged protein(r*Ts*-CLP) was purified by a on-column refolding procedure. Anti-*Trichinella* IgG antibodies in infected swine were detectable by indirect ELISA with r*Ts*-CLP as soon as 15–17 days post infection (dpi), but ELISA with muscle larval ES antigens(QIAGEN Cat. No. 273501) did not permit detection before 21 dpi. Four hybridoma cell strains against *Ts*-CLP were obtained by screening with the indirect ELISA. Blocking ELISA showed that three of them could be blocked by *T. spiralis* positive serum. Indirect immunofluorescence showed that the McAbs could combine with the natural *T. spiralis*, and native *Ts*-CLP localised to the stichosome. *Ts*-CLP liner B-cell epitope recognized by McAbs was determinated by overlapping fragments. *Ts*-CLP could be considered as a potential early diagnostic antigen for trichinellosis. The McAbs as competitive

antibodies provided foundation and technical support for the rapid diagnosis and monitoring *Trichinella* infections.

Keywords: *Trichinella spiralis*, Cystatin, Monoclonal antibody, Serodiagnosis

0086 Activity, infectivity and antibody dynamics response against *Trichinella spiralis* in experimentally infected pigs

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The objective of the present study was to investigate the activity, infectivity, and antibody dynamics in experimentally infected pigs with *Trichinella spiralis* (*T. spiralis*). A total of 30 Large White pigs (three animals per group) were inoculated with 10,000 *T. spiralis* muscle larvae (ML) and sacrificed at 12-21 days post infection (dpi) to examined activity and infectivity of ML. Another 18 pigs

(six animals per group) were inoculated with 100, 1000 and 10,000 ML, and sacrificed and calculated the average numbers of muscle larvae per gram (lpg) at 120 dpi. The pig sera were collected at 0, 7, 9, 11, 13, 15, 17, 19, 21, 25, 30, 35, 45, 60, 90 and 120 dpi. The results showed that the larvae could not be detected in diaphragm and tongue by compression and digestion until to 16 dpi. However, the larvae were inactive and noninfectious at 16 dpi. The activity and average lpg were increased significantly with time-dependent from 17 to 21 dpi. Infection groups with an average lpg of 0.007, 28.297 and 226.178, respectively. Further, the levels and dynamics of anti-*T. spiralis* IgG and IgM antibody were measured by enzyme-linked immunosorbent assay (ELISA) based on ML excretory-secretory (ES) antigens. The IgG antibody was dose-dependent manner to generated and increased throughout the experimental period. This trend continued to increased and have peaked at 90 dpi and decreased thereafter. The IgG antibody isotype IgG1 was significantly higher than IgG2a, which meant that *T. spiralis* infection induced the Th2 immune response. The results indicated that the body weight of infected pigs was significantly increased than control group. These data suggested that obesity may be associated with immunological abnormalities in *T. spiralis* infection. The IgM antibody was generated at early stage of infection with the earliest detection occurring at 7 dpi by ELISA, which might be applied to early stage diagnosis of trichinellosis.

Keywords: *Trichinella spiralis*, Pig, Excretory-secretory antigens, IgG, IgM.

0078 Filtration of *Trichinella* larvae using a microfluidic device

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The Food and Agriculture Organization of the United Nations (FAO)/WHO in 2014 ranked the foodborne parasites where *Trichinella spiralis* was ranked the seventh among them. The European Network for Foodborne Parasites (FA1408) also prepared a study to rank foodborne parasites.

The detection of *Trichinella* larvae at the slaughterhouse is regulated by the ISO/IEC 17025:2005 international standard for quality assurance system. The international guideline EA-04/10:2002 provides an additional guidance for that. The current standard in slaughterhouses can cause false negative results due to human mistakes. We would like to develop an automated diagnostic tool to detect *Trichinella* larvae using microfluidics.

In laboratory protocols generally, the detection of the foodborne parasites has been overtaken by sample preparation and the enrichment of pathogens. The microfluidic devices can also integrate sample pretreatment steps to concentrate pathogens from biopsy, liquor, stool, or samples for serological examination.

We have developed a microfluidic filter to detect *Trichinella* larvae from artificial digested *Trichinella* infected mouse meat. The analytical platform consists of a syringe pump to inject the sample, the microfluidic device and a classical light microscope for the optical evaluation. The microfluidic device has one inlet, a sequential filtration unit, which traps the nematodes and one outlet. The device consists of two sequential filter blocks to further enhance the filtration efficiency: a V-shaped filter and a circularly arranged microcapillary filter block.

We tested the design with varying microcapillary widths. We can achieve a 98.6% filtration efficiency of *Trichinella* larvae with 0.05 standard deviation. The isolated nematodes can be manipulated to next-stage analysis (e.g., genetic analysis, drug screening, enzymatic reactions).

0069 Distribution of *Trichinella spiralis* larvae in muscle of naturally infected pigs

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Trichinellosis is caused by the parasitic round-worm Trichinella spp. There are 8 known species and four genotypes of genus Trichinella. They occur in more than 150 animal species worldwide. The major source of Trichinella for humans is pigmeat and wild boars meat. The aim of the study was to assess the presence of larvae in striated muscles of naturally infected animals. Fifty six *Trichinella* positive pig's carcasses, were collected from slaughterhouses, or from *Trichinella* suspected farms. All pigs were slaughtered under control of official Veterinary Officer in approved slaughterhouses. Examined pigs were raised indoor in uncontrolled housing conditions. Pigs were raised in Podlaskie, Wielkopolska, Kujawsko-Pomorskie and Zachodniopomorskie province. All pigs were subjected to muscle examination by digestion method according to Commission Regulation (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for *Trichinella* in meat. Samples weighting 100g of muscle were taken parallely from left and right sight of carcasses. In general 17 muscles samples from each side were taken from each animal plus tongues and *trachea*. Results were presented as larvae per gram (LPG) respecivelly: Mean/Median/Max. and Min. For the left side: *corpus linguae*: 12,5/0,21/223,4/0, *m. masseter*: 7,4/0,1/106,8/0, *m. brachiocephalicus*: 3,34 /0,06/42,5/0, *m. intercostales*: 7,22/0,06/86,68/0, *m. obliquus externus*: 6,1/0,12/99,1/0, *m. psoas major*: 5,17/0,07/81/0, *m. longissimus dorsi*: 2,94/0,05/42,1/0, *m. gluteus superficialis*: 6,93/0,07/81,1/0, *m. quadriceps*: 5,21/0,04/67,7/0, *m. trapezius:* 7,3/0,03/117,4/0, *m. flexor digitorum profundus*: 7,6/0,18/110,2/0, *m. supraspinatus*: 7,5/0,08/129,6/0, *m. subscapularis*: 7,8/0,1/124,1/0, *m. triceps*

brachii: 4,8/0,12/73,6/0, *biceps brachii*: 5,5/0,03/70,3/0, *m. biceps femoris*: 4,7/0,04/79,8/0, *diaphragma pars sinistra*: 22,56/0,16/321,43/0, *trachea*: 8,0/0,34/88,63/0. From the right side: *m. masseter*: 8,1/0,17/130,8/0, *m. bachiocephalicus*: 5,16/0,03/69/0, *m. intercostales*: 4,65/0,11/53,3/0, *m. obliquus externus*: 4,47/0,07/57,3/0, *m. psoas major*: 4,87/0,04/89,1/0, *m. longissimus dorsi*: 2,16/0,12/21,78/0, *m. gluteus superficialis*: 5,79/0,06/82,2/0, *m. quadriceps*: 3,68/0,12/48/0, *m. trapesius*: 7,07/0,09/106,2/0, *m. flexor digitorum profundus*: 7,08/0,05/88,6/0, *m. supraspinatus*: 7,69/0,1/130/0, *m. subscapularis*: 6,72/0,14/78,9/0, *m. triceps brachii*: 10,8/0,1/214,3/0, *biceps brachii*: 5,08/0,1/84,3/0, *m. biceps femoris*: 5,97/0,12/85,2/0, *diaphragma pars dextra*: 18,7/0,2/295/0. The initial dose was unknown it has to be highlighted that this study was done on naturally infected pigs, and according to our knowledge it's a first study on such large group of naturally infected animals. *Trichinella* larvae were found in 12 out of 17 of digested samples of trachea. The presence of these larvae in trachea indicate that organs from infected swine cannot be assured to be free of *Trichinella* larvae. No significant difference was observed between left and right side.

0017 Validation of the PrioCHECK Trichinella AAD Kit for the detection of larvae in pork, and preliminary studies on horse meat and wildlife tissue

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The PrioCHECK Trichinella AAD Kit (TAAD) is a recently developed artificial digestion assay for the detection of *Trichinella* larvae in the muscle of infected animals. It uses an alternative enzyme, serine protease, and no hazardous substances such as HCl or pepsin. Activation of the enzyme requires an elevated digestion temperature of 60°C which kills the parasite

and reduces the risk of contaminating the environment with *Trichinella*. Compared to the pepsin-HCl method, digestion using the TAAD has several advantages, such as safety, standardization, quality, availability, and time. To assess the Kit's suitability for *Trichinella* testing, and to validate its performance relative to the conventional pepsin-HCl digestion method, several comparative studies were conducted using meat from domestic food animals and wildlife species. Multiple muscle samples were collected from diaphragm, tongue, masseter, loin or foreleg of adult pigs, horses, wild boars, bears or wolves. Samples were naturally infected or spiked with 3, 4, 5, or 25 encapsulated larvae of *Trichinella spiralis*. A total of 320 100 g spiked pork samples were used to validate and compare the diagnostic proficiency of the Kit with the pepsin-HCl digestion method. Analysis of the data generated showed that both methods are capable of consistently detecting *Trichinella* in pork samples which contained as few as 3 larvae per 100g of meat. In conclusion, the TAAD performed satisfactorily according to various international guidelines for the detection of *Trichinella* infection in four muscle types of pork samples.

Keywords: *Trichinella*, diagnostic test, AAD Kit, validation, pork.

POSTER PRESENTATIONS

0033 New reagent products for improved handling and dispensing of HCl and pepsin in the gold standard digestion method for the detection of *Trichinella*

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Hydrochloric acid and pepsin enzyme are essential reagents in the artificial digestion reference method for the detection of *Trichinella* in meat. This gold standard test method is prescribed and used globally as the primary tool in programs for the control of *Trichinella* in pork production and international trade. In recent years, *Trichinella* testing laboratories have been faced with increasing challenges regarding the quality, safety or availability of one or both of these test reagents. To address these challenges, two new products were developed for improved handling and dispensing of HCl and pepsin in preparing the digest solution. Prototype products were produced and various assessment and verification studies have been performed, including tests for performance, ruggedness, and stability. Key benefits of the new reagent products include a) consistent performance, b) improved quality assurance, c) mitigation of hazardous risks, d) time savings, and e) convenience. A ring trial validation study will be conducted in several *Trichinella* national reference labs to confirm the utility and effectiveness of the reagent products. Details of the products and the results of the studies confirming their benefits and advantages, relative to the use of conventional reagents will be discussed.

0055 Comparison of the diagnostic performance of an in-house and commercial ES ELISA for the detection of *Trichinella* infection in pigs

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Indirect enzyme-linked immunosorbent assay (ELISA) with excretory-secretory (ES) antigen of *Trichinella spiralis* is recommended by the International Commission on Trichinellosis (ICT) and the World Organisation for Animal Health (OIE) for surveillance and epidemiological studies in pigs. Our efforts to optimize and standardize ES antigen production and ELISA protocols led to improved diagnostic performance of an in-house ES ELISA. We compared the performance of our assay to that of a commercial ES ELISA kit (PrioCHECK™ Trichinella Antibody ELISA Kit, Thermo Fisher Scientific) using sera from commercial pigs, presumably *Trichinella*-free, from the national herd (n = 918), and from pigs experimentally infected with five different *Trichinella* spp. (n = 88). Both assays correctly identified positive and negative sera resulting in 100% diagnostic sensitivity and specificity. However, our ES ELISA exhibited a higher discriminatory power, as indicated by markedly better separation of absorbance values of positive sera from those of samples collected from the negative pig population. Furthermore, significantly higher serial dilutions of sera from pigs experimentally infected with *T. spiralis*, *T. pseudospiralis*, *T. britovi* and *T. nativa* tested positive by the in-house ES ELISA, confirming a higher analytical sensitivity of this assay. Use of this in-house ES ELISA will support the demonstration of negligible risk of *Trichinella* infection in Canadian commercial swine.

0008 Detection of Anti-*Trichinella* IgG In Dogs In Turkey. A Preliminary Study

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Background: Similar to other Muslim countries, in Turkey there is not much awareness of *Trichinella* infections although several human outbreaks and sporadic cases have been reported since the 1970s. Consequently, there are no many studies on the epidemiology of these zoonotic infections. Hunting activities play an important role in *Trichinella* epidemiology since wild carnivorous and omnivorous animals are the most important reservoirs of these parasites. It has been suggested that the circulation of *Trichinella* spp. among wildlife can be monitored by testing sera from hunting dogs which act as sentinel animals. The aim of the present work was to evaluate the *Trichinella* circulation in the area of Eğriöz and Cennetler Çiftliği (Kütahya, Turkey) by the serological detection of anti-*Trichinella* IgG in hunting dogs.

Methods: Serum samples were collected from 8 wild boar hunting dogs and 5 shepherd dogs. Sera were tested by ELISA using *T. spiralis* excretory/secretory antigens.

Results: Out of 13 dog sera, three hunting dogs and three shepherd dogs (46%) tested positive for IgG anti-*Trichinella*.

Conclusion: These preliminary data suggest that that *Trichinella* is circulating in the studied area.

Keywords: Trichinellosis, Turkey, Dogs, Serology

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0098 Evaluation of a novel competitive ELISA for detection of *Trichinella* infection in swine experimentaly infected with different species of *Trichinella* genus

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A Competitive enzyme-linked immunosorbent assay (c-ELISA) for detection of *Trichinella*-infection, based on application of monoclonal 7C2C5 antibodies specific for an epitope unique to the muscle larvae of the *Trichinella* genus, was developed. The *Trichinella* c-ELISA, is able to detect *Trichinella*-specific antibodies in the sera from humans and swine infected with *T. spiralis*, with the potential use for sera from other animal hosts (confirmed for horses) and other species of *Trichinella* (confirmed for *T. britovi*). The test is potentially universal since employs a single antibody, mAb 7C2C5 (HRP labeled), as both the competing and detecting reagent, which allows the detection of specific antibodies irrespective of their isotype or host origin.Therefore, the aim of this study was to examine the possibility of using *Trichinella* c-ELISA in detection of infection caused by different *Trichinella* species, on the model of experimentally infected swine.

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PATHOGENESIS AND IMMUNOLOGY

ORAL PRESENTATIONS

0016 *Trichinella spiralis* excretory-secretory products decrease the level and activity of matrix metalloproteinase 9 (MMP-9) on the model of experimental autoimmune encephalomyelitis in DA rats

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Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes involved in numerous physiological and pathological processes. Gelatinases (MMP-2 and MMP-9) play an important role in inflammatory processes, and this role is also recognized in the pathogenesis of autoimmune diseases such as experimental autoimmune encaphalomyelitis – EAE. It has also been shown that during the acute phase of *Trichinella spiralis* muscle invasion, accompanied by myositis, there is a significant increase in levels of MMP-2 and MMP-9, suggested markers of inflammation. Our investigation of the mechanisms underlying amelioration of EAE by *T. spiralis* muscle larvae excretory-secretory products (ES L1) included monitoring of levels and activities of gelatinases and tissue inhibitory of MMPs (TIMP1) in sera samples of treated animals. Experimental model included DA rats treated with ES L1 (control group), encephalitogen (EAE group) or with ES L1 prior to induction of EAE (ES L1 + EAE group). After EAE induction, the animals were sacrificed on day 8 (inductive phase), day 15 (effector phase) and day 28 (recovery phase).

Treatment with ES products reduced significantly the levels of TIMP-1 in ES L1+EAE group, particularly on day 8, compared to EAE group, whereas

on day 28 the levels were also reduced, but without reaching the statistical significance this protein.

The obtained results indicate the existence of statistically significant differences in the activity of MMP-9 between EAE group and ES L1 + EAE group, namely total and pro -MMP9 were significantly lower in effector phase (day 15) of the disease. There was a correlation between severity of EAE and total- and pro-MMP9 levels. Treatment with ES L1 did not alter the level and activity of MMP-2. Disease amelioration by ES L1 appeared as a consequence of lower production of pro-inflammatory IL-17 and IFNγ, and elevated production of anti-inflammatory IL-4 and IL-10.

We assume that this change in cytokine production influenced the levels of MMP-9.

0025 Dynamics of lymphocyte subpopulations in the small intestine of mice treated with probiotic bacteria and infected with *Trichinella spiralis*

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The intestinal phase is critical for trichinellosis. The protective immunity against *Trichinella spiralis* is T cell-dependent. Intraepithelial and *lamina propria* CD4+, CD8+ T lymphocytes, and CD19+ B lymphocytes from mice treated with probiotic bacteria and infected with *T. spiralis* were evaluated by flow cytometry. Probiotic strains of different origin (*Enterococcus faecium* CCM8558, *Enterococcus durans* ED26E/7, *Lactobacillus*

fermentum CCM7421, *Lactobacillus plantarum* 17L/1) were administered daily in dose of 10^9CFU/ml in 100 µl and mice were infected with 400 larvae of *T. spiralis* on 7th day of treatment.

L. fermentum CCM7421 and *L. plantarum* 17L/1 increased numbers of helper CD4+T cells in the epithelium and cytotoxic CD8+ T cells in the *lamina propria* on 7th day of administration (before parasitic infection). *T. spiralis* infection caused a significant inhibition of examined lymphocyte subpopulations from 5 to 25 days post infection (p.i.). Lactobacilli restored the CD4+ T cell numbers in the epithelium and *lamina propria* on the level of healthy control from day 11 p.i. All strains stimulated the numbers of CD8+ T cells in infected mice, but in comparison to control, CD8+ T cells were reduced in the epithelium until day 25 p.i. and in the *lamina propria* only on day 5 p.i. An inhibition of B cells (CD19+) in the small intestine after *T. spiralis* infection was not affected by probiotic therapy till day 25 p.i., but a stimulation of B cells was found after treatment with *E. durans* ED26E/7 and *L. fermentum* CCM7421 on day 32 p.i.

The obtained results confirmed the strain-specific immunomodulatory effect of probiotic bacteria. The greatest immunomodulatory potential on the gut CD4 and CD8 T lymphocytes during *T. spiralis* infection was induced by *L. fermentum* CCM7421 and *L. plantarum*17L/1. Strains *E. faecium* CCM8558 and *E. durans* ED26E/7 activated only cytotoxic CD8 T cells in the *lamina propria.* The changes in intraepithelial and *lamina propria* lymphocyte subpopulations after probiotic therapy indicate a positive modulation of the gut immunity in *T. spiralis* infection and perspective use of tested probiotic strains in therapy of trichinellosis. The activation of the T cells in the small intestine of mice infected with *T. spiralis* can contribute to worm expulsion from the gut and stimulate the anti-parasitic immune response also in the muscle phase of infection.

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0045 Regulation of Mouse Type 2 Immune Response Induced by the Thioredoxin Peroxidase-2(TPX2) from *Trichinella spiralis*

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Trichinella spiralis is an intracellular parasitic nematode that can infect many hosts including humans and causes trichinellosis. The enteric phase of *T. spiralis* infection generally induces a Th1/Th2 mixed response, which becomes type 2-biased during the systemic phase. Thioredoxin peroxidases from trematode can induce type 2 immune responses, but the function of thioredoxin peroxidases from *T. spiralis* in regulating type 2 immune response has not been identified. In our previous study, three members of the TPX family were cloned from *T. spiralis* muscle larvae (ML). In this study, we further confirmed the function of TsTPX2 in regulating type 2 immune response. Immunolocalization with anti-TsTPX2 mouse sera confirmed that TsTPX2 protein was strongly expressed on the surface and within the body of *T. spiralis* day 3 adults (Ad3). Immunization with recombinant TsTPX2 produced higher levers of Th2 (IL-4) and lower Th1 (IFN-γ, IL-6) cytokines; the percentage of CD4+T cells increased and the percentage of CD8+T cells declined at the same time. Moreover, the expression of characteristic molecular of AAMs (Arg-1, Mrc-1) was up-regulated and the expression of characteristic molecular of CAMs (iNOS) was de-regulated when RAW264.7 cells and peritoneal macrophages were stimulated respectively with recombinant TsTPX2. Taken together, results from our study suggested that the thioredoxin peroxidase-2 from *T. spiralis* can activate macrophages by the

alternative pathways *in vitro* and induce a type 2-biased immune response *in vivo*.

Keywords: *Trichinella spiralis*, thioredoxin peroxidase-2, type 2 immune responses, AAMs

0006 Evaluation of *Trichinella spiralis* larvae extract as an inhibitor of antiproliferative effect on human breast cancer cell culture - MCF-7

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The antiproliferative effect of the extract from the muscle larvae of *Trichinella spiralis* on the culture of human breast cancer cells MCF-7 was assessed. The following extract concentrations were used for testing: 12.5; 25; 50; 100; 250; 500 pg/ml, 1 mg/ml and 2 mg/ml. Visual examination carried out 24 hours after application of the Trichinella spiralis protein extract to the experimental wells showed comparability of the tumor cell culture at an extract dose from 12.5 up to 250 pg/ml. After 72 hours of cultivation at an extract dose of 1 mg/ml and 2 mg/ml, inhibition of proliferative activity and a decrease in the number of viable cells of 2.0 and 2.3 times were noted. A decrease in the total number of tumor cells was observed at an extract concentration of 500 pg/ml and 1 mg/ml. There were also morphological changes in the cells in the form of granulosity in the cytoplasm at an extract dose of 2 mg/ml. Subsequent follow-up carried out on the 6th day after application of *Trichinella spiralis* protein extract to the culture of tumor cells showed that 100% cell death occurred at the maximum tested concentration

of 2 mg/ml, and on the 7th day after application of the test extract to the wells markedly reduced proliferative activity of tumor cells at a concentration of 500 pg/ml and manifestation of cytostatic action at a dose of 100 pg/ml were noted.

The obtained results are convincing proof of the presence of antitumor substances in *Trichinella spiralis* having antiproliferative effect on growth and development of tumors, and inhibiting metastasis. In this regard, further research is needed in this direction. It will bring us closer to creating specific preventive medications against a number of oncological diseases.

0061 Serine protease inhibitor of *Trichinella spiralis* induced a process of early anti-inflammatory immune response dependent on macrophages alternative activation *in vivo*

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Trichinella spp., as one of most important food-borne parasites, was routinely recognized for its ability to regulate the host's immune response by secreted immune regulator molecules. According to the previous research, a serine protease inhibitor (serpin) from *Trichinella spiralis*, named *Ts*-Serpin, was identified with the property of inducing alternative activation of murine macrophage cell line (J774A.1) *in vitro*. In this study, *Ts*-Serpin was reselected to further investigate its immunoregulatory properties *in vivo*. The results showed that, distinctive inoculation methods of recombinant *Ts*-serpin (intraperitoneal and intravenous injection) could induce different early immune response *in vivo*, independent of T cells proliferation. Meanwhile, increasing levels of IL-10 and TGF-β appeared soon (3 days post injection) by intraperitoneal injection of r*Ts*-serpin rather than intravenous injection. At the cellular level, it was found that r*Ts*-serpin could induce alternative activation of bone marrow-derived macrophages (BMDMs). Similarly, the regulatory properties of r*Ts*-serpin could also be detected in the TNBS-induced inflammatory bowel disease (TNBS-IBD) model by preventing intestinal damage and reducing the levels of pro-inflammatory cytokines. Furthermore, adoptive transfer of r*Ts*-serpin-induced alternative activated BMDMs showed a similar therapeutic effect in TNBS-IBD model. In general, it was preliminarily demonstrated that *Ts*-serpin could induce an anti-inflammatory immune response by inducing alternative activation of macrophages in the early stage of innate immunity *in vivo*. And this property may have prospects in the treatment of autoimmune diseases.

Keywords: *Trichinella spiralis*, Serine proteinase inhibitors, Alternatively activated macrophages, Inflammatory bowel disease

0046 The NLRP3 play a critical role in development of Th2 cell-mediated protective immune response against *Trichinella spiralis* infection

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Inflammasomes are involved in immune defense against infectious pathogens, but less is known about the role of NLRP3 in *Trichinella spiralis* (*T. spiralis*) infection, which characteristically induces T-helper 2 cell (Th2) immune repose. In this study, we investigated the roles of NLRP3 in the protection against *T. spiralis* infection by triggering Th2 immune response. Firstly, we showed that the level of NLRP3 expression in $CD4^+$ T cells was significantly increased at 7 and 21 days post infection of *T. spiralis*. Compared to wild-type (WT) $CD4^+$ T cells, the expression of IL-4 mRNA was reduced in $NLRP3^{-/-}$ $CD4^+$ T cells, meanwhile the expression of IFN-γ mRNA in $NLRP3^{-/-}$ $CD4^+$ T cells was similar with $CD4^+$ T cells from WT mice. Consistently, ELISA and flow cytometers results showed that $NLRP3^{-/-}$ $CD4^+$ T cells secreted less IL-4 than did $CD4^+$ T cells from WT mice, whereas IFN-γ secreted by $NLRP3^{-/-}$ $CD4^+$ T cells is similar to those secreted by WT $CD4^+$ T cells. In addition, we

observed a significant reduction of IL-4 by ELISA in NLRP3$^{-/-}$ mice compared with WT mice at 7, 21 and 35 days post infection, respectively. Furthermore, we demonstrated that adult worm survival was substantially prolonged and muscle larvae burden was increased in NLRP3 deficient mice compared to WT mice. Our results first reveal that NLRP3 play a role in the development of Th2 response during *T. spiralis* infection and NLRP3 may be involved in host protection against *T. spiralis*.

Keywords: *Trichinella spiralis*, NLRP3 inflammasome, CD4^{+} T cell, Th2 immune response, protective immune

0077 Immunogenicity of recombinant multi-cystatin-like domain protein from *T. britovi*: *In Vivo* study in mouse model

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Trichinellosis is important parasitic zoonosis, caused by consumption of raw and undercooked meat, containing infective larvae of the *Trichinella* parasite. From several species of *Trichinella*, that occur in European area, *T. britovi* is one of the most common but relatively poorly investigated. Therefore the broad study of *T. britovi* antigens and its immunological interactions with host would be advisable, as this parasite can infect a wide variety of hosts, including humans. In our previous study we used immunoproteomic approach and identified multi-cystatin-like domain protein (CLP) as an immunologically active protein from *T. britovi* muscle larvae recognized by sera from infected pigs. This protein was selected for cloning and expression in *Pichia pastoris* yeast system. Then, obtained 47 kDa recombinant protein was used for immunization of mice. Immunological potential of rCLP was analyzed by detection of IgG, IgG1, IgG2a antibodies and

selected cytokines (IFNγ, IL-2, IL-4, IL-10), secreted by stimulated splenocytes isolated from immunized and/or infected animals. Furthermore, muscle larvae burden was measured at the end of experiment (48 days post infection), as an indicator of potential protective role of rCLP.

This study, demonstrates that *T. britovi* rCLP elicited high titer antibodies level, visibly affected the profile of cytokines secreted by stimulated splenocytes, in comparison to naïve animals, and reduced the number muscle larvae. It makes rCLP promising candidate for subsequent studies and future usage in immunodetection and vaccination against *T. britovi* parasites.

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POSTER PRESENTATIONS

0047 *Trichinella spiralis* can induce neutrophils to release NETs

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As the most important innate immune cells against exotic pathogens, neutrophils can release extracellular traps (ETs) composed of DNA skeleton and granule proteins, in addition to the traditional function of phagocytosis and cytokines secretion. Neutrophil extracellular traps (NETs) play a key role in capturing and killing pathogens in vivo. A variety of species and compounds have been reported to induce neutrophil to release NETs so far. But only parasitic nematodes have not been reported to cause neutrophil to release NETs until now. In this study, adult worms of *Trichinella spiralis* (*T. spiralis*) was used to co-culture with polymorphonucleocytes (PMN) extracted from mouse bone marrow to see if nematodes can also induce neutrophils to

release NETs. PMN was extracted to verify its purity and function firstly. We used flow cytometry and nuclear staining to test the purity of PMN, and the results showed that the purity of PMN reached about 90% and could be used in the subsequent experiments. PMN was then stimulated with PMA and LPS, which are widely regarded as reagents that cause neutrophils to release NETs, and the results showed that PMN we extracted could release NETs with H3, MPO and elastase on DNA skeleton after stimulation. The DNA content in cell culture supernatants increased with the increase of time and dose of PMA and LPS, which indicated that the PMN extracted has normal cell function. Subsequently, we co-cultured *T. spiralis* adults (100 worms/dish) with PMN for 3 h and stained with Hoechst 33342 / Sytox Green. Under laser confocal microscopy, we observed that live adult worms were able to induce PMN to release NETs, while dead worms could not.

Keywords: *Trichinella spiralis*, neutrophils, co-culture, NETs

0076 Regulation of host immune cells and cytokines expression induced by *Trichinella spiralis* infection

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Trichinella spiralis can cause immunoregulation during the early phase of infection. However, the previous results are still insufficient for a full

understanding of the phenomenon and mechanism. Immune cells and cytokines profiles were investigated by Meso Scale Discovery (MSD) and flow cytometry of mice infected with *T. spiralis* in this study. The MSD results of spleen showed that Th1 immunity was inhibited from 6 h to 6 days post-infection (dpi) and the level of Th2 immune response was significantly increased at 6 dpi. The mesenteric lymph node was showed a Th1/Th2 mixed immune response from 3 to 6 dpi with a downtrend of Th1 at 6 dpi. Flow cytometry analysis showed that the ratio of $CD4^+/CD8^+$ was significant decrease at 6 h after infection, suggesting that the host was in a significant immunosuppressive state at this time. In addition, the proportion of Th1 cells of T cells was decreased significantly at 6 h after infection, the ratio of Th2 was increased markedly, Th17 and Treg were showed no significant changes, indicating that Th1 immunity was significantly inhibited at 6 h after infection, and a hybrid immune response based on Th2 type was presented from 30 h to 6 dpi. Moreover, immunoregulation effects during the study period provide a better understanding of the development of immune response induced by *Trichinella* infection and even have a great impetus to the occurrence of allergic diseases, autoimmune diseases, and tumors.

Keywords: *Trichinella spiralis*, Meso Scale Discovery, cytokines, immunoregulation

0027 Resveratrol reduces oxidative damage and inflammation in mice infected with *Trichinella spiralis*

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Trichinellosis is a serious food-borne zoonotic infection of cosmopolitan distribution. Currently, the treatment of trichinellosis is far from

ideal. Given the important role of oxidative stress and immune-mediated inflammation in its pathogenesis, this study was designed to evaluate the possible protective effects of resveratrol (RSV) during the intestinal and muscular phases of *Trichinella spiralis* infection in mice. Oral administration of RSV in a dose of 20 mg/kg once daily for two weeks resulted in significant reduction of both adult and larval counts; significant improvement of the redox status in muscles and small intestines; significant reduction of IL-4, pentraxin 3, and vascular endothelial growth factor (VEGF) expression; and mitigation of small intestinal and muscular inflammation. In conclusion, this study introduced RSV as a promising agent in the treatment of trichinellosis, and clinical studies are needed to explore its efficacy in human trichinellosis.

Keywords: *Trichinella spiralis*; Resveratrol; Oxidative stress; IL-4; PTX3; VEGF

0083 Cytokine gene expression in the gut and in the spleen of mice modulated by probiotic bacteria and *Trichinella spiralis* infection

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The host-protective immune response against *Trichinella spiralis* is mediated by T helper cytokines. Probiotic bacteria modulate also cytokine release to maintain the balance between essential and excessive activation of immune defense mechanisms. This study was focused on the effect of probiotic therapy on mRNA expression of Th1 (IFN-γ, TNF-α) and Th2 (IL-4, IL-5, IL-10) cytokines in the small intestine and the spleen of mice with *T. spiralis* infection. Two bacterial strains *Enterococcus faecium* CCM8558 and *Lactobacillus fermentum* CCM7421 were administered daily in dose of

10^9CFU/ml in 100 µl *per os* and mice were infected with 400 larvae of *T. spiralis* on 7th day of treatment.

Both probiotic strains prevented the growth in IL-10, IFN-γ, and TNF-α gene expression in the intestinal tissue on days 5 and 18 post infection (p.i.). *E. faecium* CCM8558 stimulated mRNA for TNF-α and IL-10 on day 11 p.i. The expression of IL-10 in the gut was downregulated during the muscle phase of the infection in treated mice. Probiotic therapy reduced IL-4 and IL-5 gene expression in the gut till day 18 p.i. The immunomodulatory effect of probiotic therapy in the spleen of infected mice was demonstrated by *E. faecium* CCM8558 with a significant increase in IFN-γ, TNF-α and IL-10 mRNA levels on day 18 p.i. (Th1/Th2 immune response). The early muscle phase of trichinellosis in the spleen (days 11. and 18. p.i.) was accompanied with increased IL-5 expression caused by *L. fermentum* CCM7421.The levels of IL-5 mRNA in the spleen were significantly inhibited by probiotic strains later, on day 25 p.i. The strain *L. fermentum* CCM7421 caused a significant reduction in IL-4 gene expression in the spleen on days 18 and 25 p.i., and *E. faecium* CCM8558 on day 25 p.i.

The obtained results suggest the anti-inflammatory effect (down-regulation of IFN-γ and TNF-α gene expression) of both probiotic strains in the gut during the intestinal phase of trichinellosis and *E. faecium* CCM8558 regulated the immunological balance in the early muscle phase. The gene expression of IL-5 was associated with the intestinal phase and was inhibited by probiotic therapy. The IL-4 gene expression was reduced by probiotic strains during the muscle phase, but *L. fermentum* CCM7421 increase its expression in the intestinal phase. The probiotic strains used different modulating ways to protect the host against parasite infection and their modulating effect is strain-specific.

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0081 Experimental Infection with *Trichinella spiralis* Tibet strain in Hezuo Pig

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Trichinellosis, caused by *Trichinella,* is a serious foodborne parasitic zoonosis. Pork and other animal meat are identified as the most important source of infection for humans. Hezuo pig is a sort of primitive local pig, which is from natural domestication and artificial selection. Because of the free-range systems, Hezuo pig is liable to be attacked by *Trichinella* spp. The aim of the present work is to study the infectivity of *T. spiralis* Tibet strain to Hezuo pig and the changes of cytokine levels in sera. A total of 8 Hezuo pigs were divided into 4 groups, including three experimental groups and one control group. The pigs in the experimental groups were inoculated with 200, 2000 or 20,000 muscle larvae of *T. spiralis* Tibet strain respectively. The blood samples were collected at days 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 100 and 107 post-infection, and the sera were isolated to evaluate the anti-*Trichinella* IgG kinetics by ELISA. The results of ELISA showed that the level of antibodies was corresponded with the inoculum dose before the days 56 post-infection. Ten cytokines were detected by microarray in the serum, which include four Th1 cytokines (IFN-γ, IL-1β, IL-8, IL-12), three Th2 cytokines (IL-4, IL-6, IL-10), two Th1/2 cytokines (GM-CSF, TNF-α) and one Th3 cytokines (TGF-β1). All the cytokines were detectable in all the samples. Of all the cytokines, IL-12 demonstrated the highest concentration compared with other cytokines and peaked on day 42 post-infection. The four kinds of cytokines had a low concentration in the early period of infection. But after the day 42 post-

infection, almost all the cytokines maintained in a high level. At necropsy, muscles from eight organs including diaphragm, tongue, masseter, intercostal, psoas, gluteus, foreleg and hind leg were digested artificially to evaluate the muscle larvae burden. There was a positive correlation between the infective dose and the mean larval recovery rate, and the diaphragm and tongue were identified as predilection muscles. The experimental pigs receiving 20,000 larvae showed a maximum of 315 lpg in diaphragm and a minimum of 52 lpg in intercostal, while the pigs receiving 200 larvae showed a maximum of 10 lpg in diaphragm and a minimum of 1 lpg in intercostal. It is the first record of experimental infection in Hezuo pig with *Trichinella spiralis* Tibet strain.

Keywords: *Trichinella spiralis*, Hezuo Pig, necropsy, cytokines, IgG antibody

0070 Evaluation of the protective effect of various immunostimulating drugs in experimental trichinosis of mice

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Trichinosis is a parasitic disease caused by roundworms of the Trichinella type. Geographic distribution – worldwide, this is one of the most widespread zoonotic pathogens on the world. Now, the immunostimulatory drugs in recent years are widely used for parasitic disease in veterinary medicine. In our study we evaluated the protective effect of meglumine acridonacetate (cycloferon), roncoleukin and azoximeri bromidum against experimental trichinosis, that can affect the muscle phase of the parasite *Trihinella spiralis* in mice. The meglumine acridonacetate (cycloferon) is acridone acetic acid derivative, low molecular weight inducer of interferon synthesis with pronounced interferonogenic activity. The roncoleukin is

recombinant interleukin-2 human (rIL-2), is a complete structural and functional analogue of endogenous interleukin-2, isolated from cells of the recombinant strain of the yeast *Saccharomyces cerevisiae*. The azoximeri bromidum, is a polymer, a combined product with immunomodulating, detoxifying and antioxidative action.

We used 40 mice weighting 16-18 g, divided into 4 groups of 10 animals in each. The first group was injected with cycloferon intramuscularly in the dose of 2.16 mg/mouse; the second group was injected with roncoleukin subcutaneously in the dose of 100 U/mouse; the third group was injected with azoximeri bromidum in a dose of 0,004 mg/mouse; the four - control group was injected with 0.9% NaCl. The all drugs were injected in 0,2 ml sterile saline twice with an interval of 48 hours. After a 48 hours regimen, the groups were infected by *T. spiralis* larvae in the dose of 80 ± 5 larvae/mouse. After 90 days of incubation, the mice were euthanized and dissected for evaluation. Carcasses of mice were subjected to digestion in artificial gastric juice. Muscle larvae *T. spiralis* were collected and calculated based on statistical methods. Analysis of the data indicates that in the experiment in the application of these drugs has been significant protective effect.

The number of *T. spiralis* larvae detected in animals was: in the first group was injected with cycloferon - 733.5±25.1; in the second group was injected with roncoleukin - 869.5±16.61 and in the third group was injected with azoximeri bromidum -142.5±11.1 respectively. In control group number of *T. spiralis* was 4485±430.6 larvae/mouse. This was 6.1, 5.2 and 31.5 times less than in the mice of control group.

Based on this, we consider it expedient to continue the study of mmunostimulatory drugs in the complex immunoprophylaxis of trichinosis as a possible component for the future creation of a vaccine.

0059 Plasmagenesis dynamics in experimental *Trichinella* infection

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Acute and chronical helminthiases lead to the development of immune responses in the host that play a leading role in all the pathological process related to these infections. Important immune processes are carried out in the spleen and bone marrow. In these organs, plasmacytic reaction and starting mitosis in cells indicates the immune activation of the host to develop immune responses. The aim of this work was to evaluate the plasmacytic reaction dynamics during *Trichinella* infection in rats. Twenty outbred white rats 5-6 months old weighting 230-250 g were infected per os with 10 *Trichinella spiralis* L3 per g of weight. Five uninfected rats with the same age and weight constituted the control group. After infection, rats were euthanized at days 4, 8, 26 and 38 p.i. The spleens were collected and stamps on microscope slides were fixed in methanol and stained with Pappenheim (Pokrovskaya et al.1965). Plasma lineage cells were counted in 50 optical microscope fields and plasmacytic reaction was evaluated. Three types of plasma cells were observed: plasmablats, immature plasma cells and mature plasma cells. Mitotic activity (the percentage of proliferating cells from the total cells count) and chromosome aberrations were evaluated in spleen and bone marrow cell populations after carmine acetate staining. Plasmacytic reaction directly depended on the *T. spiralis* development stage. High levels of blasts, mitotic and plasma cells, in particular immature plasma cells were observed in cell populations at early intestinal (4-5 days p.i.) and muscle stages (30 days p.i.).

On the contrary, during the migration stage of the parasites a decrease of plasmacytic reaction, lower than that of the control group, along with a decrease of proliferation and mitotic activities were observed. At 38-40 days p.i., a normalization of plasmocytic reaction and mitotic activity happened. At all stages of the parasite development, the numbers of cells with chromosome aberrations remained similar. The degree of immune maturation of spleen and bone marrow cells directly depended on *T. spiralis* development stage. Stimulating effect of the parasites was pronounced at early intestinal and early muscle stage, whereas suppressive effect of this parasite was evident at migrating stage.

0063 Glutathione-S-transferase omega 1: a possible role in nurse cell developement

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The glutathione-S-transferases omega (GSTO) are multifunctional enzymes, involved in cellular defense and with distinct structural and peculiar functional characteristics. They differ from other GSTs, as they lack any glutathione transferase activity, whereas they possess thioltransferase and dehydroascorbate reductase (for this reason the first which was purified, GSTO1, was previously named dehydroascorbate reductase). Moreover, GSTO1 overexpression is associated with activation of survival pathways (Akt and ERK1/2) and inhibition of the apoptotic process (JNK) (Piaggi et al., 2010). In addition, GSTO1 is required for LPS-mediated signaling in macrophages, being a pro-inflammatory marker and macrophages deficient for GSTO1 fail to underlie the LPS-triggered glycolytic switch (Board et al., 2016). Our previous studies showed that the GSTO1 is overexpressed in the nurse cell (NC) during *Trichinella spiralis* infection (Bruschi et al., 2003).

During NC formation the structural and regulatory genes of the skeletal muscle cell are downregulated and a new phenotype is acquired, which will allow parasite growth and survival.

In order to clarify the host-parasite relation on the NC formation, we evaluated by immunohistochemistry GSTO1 production after 15, 28 and 60 days from experimental mouse infection with *T. spiralis*.

The staining of the NC appeared significant already after 15 days of infection to progressively increase up to 60 days of infection, compared to the surrounding muscle. Considering the correlation between GSTO1, AKT and JNK we decided to evaluate the phosphorilation status of AKT and JNK in the NC at the corresponding times of infection. The activation pattern of AKT is similar to that of GSTO1 overexpression. JNK, on the other hand, was not phosphorylated. To understand the molecular mechanisms of these observations obtained *in vivo*, we performed *in vitro* experiments adding 50ug/ml of excretory-secretory products (ES) from *T. spiralis* L_1 larvae, prepared as previously described (Gomez-Morales et al. 2008) to U937 cells, a human cell line established from a diffuse histiocytic lymphoma, with many monocytic characteristics that could help us to understand the effects of ES on immune cells.

Immunoblotting analysis on cells treated with ES for 24, 48 and 72 hrs,, showed a progressively overexpression of GSTO1 over the time whereas AKT was activated only after 72 hrs of treatment. In conclusion, these data show also in a parasitic infection model that GSTO1 is involved in pro-inflammatory processes, opening the way to the identification of new pharmacological targets.

STUDENT RESEARCH AWARDS

ORAL PRESENTATIONS

0107 Experimental study in wild boars infected with *Trichinella patagoniensis*, *T. pseudospiralis* and *T. spiralis*

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1. Introduction. Trichinellosis is a relevant disease for the public health in Argentina, essentially because of the hunt of wild animals, unofficial slaughterhouses and commercialization of meat products without previous diagnosis of this parasite. In order to determine another method to control and reduce the impact of this disease, the effectiveness to inactivate *Trichinella* muscle larvae (ML) with irradiation is put under study. In Argentina the present species of *Trichinella* found are *T. spiralis*, *T. pseudospiralis*, *T. britovi*, and *T. patagoniensis.* This last specie mentioned was only found in mountain cougars in this country and, limited information of this specie is known. For this reason, this study aims to determine the susceptibility, serological response, larvae distribution and irradiation effects of wild boars infected with *T. patagoniensis*, *T. spiralis* and *T. pseudospiralis* and compare them all.

2. Materials and methods. 2.1. Experimental design. Eighteen wild boars (*Sus scrofa*), 60 days of age, were used. Each wild boar was inoculated per os with 20000 larvae using a stomach tube. The genotypes employed were *T. patagoniensis* (ISS2311, from a mountain cougar), *T. pseudospiralis*

(Krivokapich et al., 2015 from a domestic pig), and *T. spiralis* (ISS1097, hybrid pig, *Landrace x Yorkshire*). The parasites were maintained in CF1 mice, and recovered by artificial digestion. Each *Trichinella* genotype was inoculated into 5 wild boars, and additionally, three animals served as uninfected control. The animals were sacrificed 19 weeks post inoculation (pi).

2.2. Larval distribution. To determine larval distribution from each wild boar, 9 muscles or muscle groups were analysed by artificial digestion. Twenty grams of muscle samples were used from: tongue, masseters, boston butt, oesophagus muscle, diaphragm, intercostal muscles, tenderloin, upper foreleg and upper hindlimb. For wild boars inoculated with *T. patagoniensis,* 100 gram samples were used. All muscles were freed from fascia and tendons, and digested using artificial digestion (Gamble et al., 2000). Recovered larvae of each muscle sample were expressed as larvae per gram (lpg).

2.3. Serology. Blood samples were weekly collected with EDTA, by jugular venepuncture. This procedure was done at weeks 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, and 19 pi. The serums were stored at -80° C until used. Serum samples were evaluated using the ELISA Kit, PrioCHECK *Trichinella* Ab.

2.4. Muscle Juice. After euthanasia, tissue samples from tongue, diaphragm, upper foreleg and upper hindlimb were collected in conical containers and frozen at -20°C for 24 hours in order to obtain the muscle juices. Afterwards the samples were let to thaw at 4°C for 18-24 h. Muscle juice samples were maintained frozen at -20°C until immunoassay was performed using an ELISA Kit, PrioCHECK *Trichinella* Ab.

2.5. Irradiation. Wild boar infected meat with *T. spiralis* and *T. pseudospiralis* were used to determine the effectiveness of irradiation to inactivate muscle larvae. Two hundred and fifty grams of muscle samples of intercostal muscles, boston butt and upper foreleg were used. These were vacuum packed and sent for irradiation treatment at the Atomic Centre of Ezeiza (CNEA – Argentine National Commission of Atomic Energy) with an alanine dosimeter with a minimum and maximum dose of 0.32 – 0.41 kGy. From each treated sample 20 g of its center were taken 24 h, 7, 14 and 21 days post-irradiation and artificially digested so as to obtain muscle larvae (L1) which were afterwards inoculated in 72 CF1 mice. All mice were inoculated per os with 300 L1, and 42 days pi were sacrificed and their carcasses were individually digested. Furthermore, three mice were inoculated with 600 L1, obtained from the irradiated and digested muscle samples, to recover and

count adult worms. They were sacrificed 72 h pi, and their intestine was removed, longitudinally opened, and cut in smaller pieces of 5 cm. This was placed in 50 ml Falcon tubes with 0.9% NaCl saline solution, to incubate at 37°C for 5 h.

2.6 Statistical Analysis. Muscle larvae recovery was analysed by Kruskal Wallis analysis of variance. ELISA readings from sera and muscle juice were compared using the Spearman rank correlation test. A repeated measures design in time was applied for the evaluation of the antibody kinetics with the three *Trichinella* species (p-value <0.05).

The present study was approved under permit number 2015/16 by the Institutional Committee for Use and Care of Laboratory animals of the Faculty of Veterinary Sciences, University of Buenos Aires(CICUAL).

3. Results. *T. patagoniensis* ML were found principally in upper foreleg, diaphragm and tongue of 3 inoculated wild boars, although larvae burden was the lowest in comparison to the larvae burden of the other *Trichinella* species employed in the present study (0.01 - 0.087 lpg). The main infected muscles for the three *Trichinella* spp considered were tongue and diaphragm. The highest larvae burden was 1812 and 134.3 lpg for *T. spiralis* and *T. pseudospiralis* respectively. Both larval burdens were from the tongue muscle. Statistical differences were found in the ML recovery from *T. spiralis*, *T. patagoniensis* and *T. pseudospiralis*.

The serological response in wild boars was detected at different times pi. The seroconversion was observed among 2- 4 weeks pi for *T. patagoniensis*, at 2 weeks pi for *T. pseudospiralis* and 3-4 weeks pi for *T. spiralis*. All animals, except the control group remained above the cutoff value until the end of the experiment (week 19 pi). Statistical differences were observed among DO values in all groups at week 2 pi, between *T. pseudospiralis* and *T. patagoniensis,* and *T. pseudospiralis* and *T. spiralis* at week 3 pi, and at week 19 pi between *T. patagoniensis* and *T. spiralis*. Significant positive correlations were demonstrated between ELISA values from sera and muscle juice originating from the four muscle groups. The correlation was independent of the origin of the muscle juice.

All L1 obtained from the muscle samples irradiated preserved the integrity of their cuticle and shown active motility. However, no adult worms nor muscle larvae of *T. spiralis* or *T. pseudospiralis* were found in the infected mice with irradiated L1.

4. Discussion. The present study recorded for the first time the experimental infection of wild boars with *T. patagoniensis* and showed the ability of this parasite to develop its cycle in this host. Nevertheless, *T. patagoniensis* had low infectivity in wild boars. Similar results were found in pigs which were infected with *T. patagoniensis.* This species appeared to be more associated to *T. nativa* and *T. murrelli* (Krivokapich et al., 2012). These two species also showed low level of infectivity in rats, pigs (Murrell et al., 2000; Pozio and Zarlenga, 2005) and wild boars (Kapel, 2001). *T. patagoniensis* was first found in a cougar in Argentina by Krivokapich, and further studies showed its high capacity to infect domestic cats (Ribicich et al., 2013). Based on these observations, carnivores may be suitable hosts for this genotype.

In wild boars infected with *T. patagoniensis*, ML were mainly recovered from diaphragm, tongue, and upper foreleg and no ML were found in masseters nor intercostal muscle from any analysed animal. Likewise a study done in pigs, showed the tongue as the main infected muscle (Krivokapich et al., 2012). Diaphragm and tongue showed similar ML distribution in wild boars infected with *T. pseudospiralis* and *T. spiralis*. Comparable results were found in pigs (Smith, 1988; Kapel et al., 1998; Kapel, 2001; Nöckler et al., 2005) and their ML distribution per muscle had no substantial differences among the species under study. Similar results were obtained by Kapel (2000); Kapel (2001); Kapel et al. (2005), in wild boars, pigs and horses. Moreover, all species analysed had showed predilection for the tenderloin and boston butt muscles.

Besides, more information about irradiation regarding its effectiveness to treat infected meat with *Trichinella* spp is needed. Nonetheless, according to the results obtained from the irradiation study, this method showed its efficacy on inactivating encapsulated and nonencapsulated *Trichinella* ML. In encapsulated species similar results were found by Gibbs et al. (1964); Brake et al. (1985); Kásprzak et al. (1994). So irradiation could be thought as a possible treatment to ensure the innocuity of meat products, but further studies should be done to assure this statement.

Furthermore, all infected wild boars seroconverted before 29 days pi. Comparable results were found in pigs inoculated with 10000 larvae (Kapel and Gamble, 2000) and wild boars with that infection dose (Kapel, 2001) of sylvatic and domestic *Trichinella* spp. In another study with SPF pigs with the

same dose of *T. spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis* all seroconverted before 40 days pi (Nöckler et al., 2005).

Infected animals with *T. spiralis* and *T. patagoniensis* showed higher antibody levels throughout the nineteen weeks of study, than wild boars infected with *T. pseudospiralis*. This was even seen in wild boars infected with *T. patagoniensis* in which no ML were found. This may be due to the fact that *T. pseudospiralis* is an unencapsulated genotype, so the stimulation of the immune system might differ, but further studies are need to understand this biological diversity (Kapel, 2001). Some differences regarding the rapid antibody response in wild boars inoculated with *T. pseudospiralis* was seen, in contrast to what was found by Kapel in 2001; in which the antibody increase was more delayed. *T. spiralis* and *T. pseudospiralis* remained in a plateau after reaching the maximum level. Similar results were seen by Kapel (2001); Kapel and Gamble (2000) and Bolas-Fernandez et al. (1992). Otherwise, for *T. patagoniensis* a decline in the antibody response was seen, nevertheless it remained always over the cutoff value. In addition, the OD value of the sera and the muscle juice showed a positive correlation. So muscles juice can also be used for epidemiological research, and it has the advantage that it can be obtained from dead animals, and can be stored for long periods of time for future use. Moreover, for *T. patagoniensis* this could be a fundamental source to use for epidemiology studies, as the larvae burden were very low in the animals under study. The role of wild boars (*Sus scrofa*) in the transmission of *Trichinella* species found in Argentine, the appropriate diagnosis methods in wild boars, the impact of infected wild boar meat, and the new knowledge about *T. patagoniensis* are essential to prevent and control this important zoonoses in this region.

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0106 Distribution and genetic diversity of *Trichinella* in Canadian wildlife: A previously undescribed species (T13) and an unexpected discovery of *T. spiralis*

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Introduction: Trichinellosis is an important food borne disease caused by nematodes of the genus *Trichinella.* Five species of *Trichinella* [*T. spiralis* (T1), *T nativa* (T2), *T. pseudospiralis* (T4), *T. murrelli* (T5) and *Trichinella* T6] have been documented in domestic and wild animals from Canada; T2 and T6 are the predominant species. Almost all human outbreaks

in the last 20 years in Canada involved *T. nativa* and *Trichinella* T6 linked to consumption of raw or improperly cooked game meat. *Trichinella spiralis* has been eradicated from commercial confinement-raised pigs and is immediately reportable to animal health authorities in Canada. There has been no evidence to support that *T. spiralis* exists in wildlife reservoirs, nor that spillover from the domestic cycle is occurring. However, gaps remain in *Trichinella* surveillance from Canadian wildlife. Therefore, we studied the distribution and genetic diversity of *Trichinella* in terrestrial mesocarnivores in Canada, using them as sentinel hosts due to their high trophic positions.

Materials and methods: Tongues/diaphragms were collected during necropsies performed on the carcasses of 469 wolverines (*Gulo gulo*, from Yukon and Northwest Territories), 39 Arctic foxes (*Vulpes lagopus*, from Northwest Territories) and 50 lynx (*Lynx canadensis*, from Quebec). Larvae of *Trichinella* spp. were recovered following artificial digestion of host tissues (Forbes and Gajadhar, 1999). Multiplex PCR was performed on DNA extracted from the larvae (5 individuals and one pool of 10 larvae per animal) (Zarlenga et al., 1999) to identify species present. Amplified DNA fragments were sequenced from mitochondrial Cytochrome Oxidase Subunit I (COI) and *Nad 5* genes as well as nuclear 5S rDNA intergenic spacer region (5S rDNA-ISR) and D3 rDNA to confirm the identity of the isolates, and to determine hybridisation and genetic diversity (Gasser et al., 2004; Krivokapich et al., 2015; Rombout et al., 2001; Franssen et al., 2015).

Results: Prevalence of *Trichinella* spp. was highest in wolverine (73%), followed by Arctic fox (18%), and lynx (10%). In both Arctic fox (7/39) and lynx (5/50), T2 was the only identified *Trichinella* species.

Of 335 isolates of *Trichinella* spp. from wolverine, *Trichinella* T6 was the predominant species (69%) followed by T2 (15%) and mixed infections (both T2 and T6, 15%). Collective 5S rDNA-ISR and COI sequence information of individual *Trichinella* larvae (1-5 individual larvae per animal) recovered from six wolverines (3 animals positive for T2 and 3 for T6 based on multiplex PCR) and five lynx (all positive for T2 on multiplex PCR) showed hybridization between T2 and T6 in four wolverines (three were T6 on 5s-ISR and T2 on COI, and one was T2 on 5s-ISR and T6 on COI). Haplotypes of the COI gene were also demonstrated in individual T2 muscle larvae from wolverine, lynx and Arctic foxes. Unexpectedly, among 42 wolverine samples determined to be *T. nativa* based on multiplex PCR, 14 were consistent with a putative

undescribed species of *Trichinella* (T13) based on DNA sequence and using a newly developed PCR-RFLP.

Interestingly, in addition to infections with *T. nativa* and T6, one wolverine was infected with T4 (*T. pseudospiralis*) and one wolverine was infected with *T. spiralis* (T1). Phylogenetic analysis of 339 base pairs of COI DNA and 400 base pairs of D3 rDNA linked the *T. pseudospiralis* isolate with those derived from the Nearctic (Vancouver Island, Canada), Palearctic or Neotropical regions, but not elsewhere in the North America (continental USA). The new *T. spiralis* isolate showed 99.6% nucleotide identity with *T. spiralis* isolates from the USA, Poland (haplotype A) and Russia compared with 99.4% and 99.2% with *T. spiralis* isolates from Belarus and India, respectively based on 772 bp of high-quality DNA sequence at the COI locus. Sixteen wolverines were retested (from the Klondike region, as well as wolverines which had ratios of dead to live larvae > 1) to determine if this was an isolated case; none of the retested animals were positive for T1.

Discussion: As compared to Arctic foxes and lynx, wolverines had high prevalence and diversity of *Trichinella* spp; they were shown to host 5 species of *Trichinella*: T1, T2, T4, T6 and T13. Wide home range and scavenging behaviour could be responsible for exposure to this wide range of *Trichinella* species in wolverines. The finding of *T. pseudospiralis* most similar to Asian or South American isolates in subarctic Canada suggests a possible role for migratory birds in long distance dispersal of this parasite.

Trichinella nativa and T6 were the most prevalent species in the subarctic with many multiple infections detected. Using only PCR either targeting nuclear gene or mitochondrial gene can miss hybrids which were not uncommon in this sample. Also, multiplex PCR can misidentify T13 as *T. nativa*. We recommend use of sequencing to confirm identification of *T. nativa* or any species of *Trichinella*, and to determine genetic diversity within this assemblage of species.

The wolverine infected with T1 (*T. spiralis*) in a subarctic region is troubling and poses a potential public health risk. The present case represents only the second report of *T. spiralis* in Canadian wildlife and the first in the subarctic from central Yukon, near the Alaska border where winter temperatures should kill *T. spiralis* in carcasses. The first verified report of *T. spiralis* in wild animals of Canada pertained to red foxes (*V. vulpus*) and coyotes (*Canis latrans*) originating from Prince Edward Island (Appleyard et

al., 1998) an area with more moderate climate. There is very little domestic swine production in the Yukon or neighboring regions of Alaska. Therefore, it is theorized this young wolverine potentially scavenged imported meat, domestic pigs raised for personal consumption, or migratory wildlife infected with the parasite. While every reasonable effort was made to thoroughly test samples, other instances of *T. spiralis* or *T. pseudospiralis* may have been missed as samples were stored frozen for extended periods of time prior to digestion. Surveys on *Trichinella* based on freshly harvested wildlife carcasses might reveal a higher prevalence than previously suspected and continued surveillance is warranted.

Conclusions: Mesocarnivores are largely harvested for their fur and are not consumed for food, with the possible exception of lynx. Therefore, detection of *Trichinella* spp. in wild carnivores does not necessarily pose a direct food safety risk to humans, but serves as an indicator of the local circulation of sylvatic species, and potentially of any circulating *T. spiralis* in local domestic and/or game animals. Although our finding of *T. spiralis* appears to be an anomaly, it nevertheless underscores the importance of surveillance in wildlife (especially sentinel species such as wolverine), regulation of importation of animals and animal products, and cross border cooperation to mitigate spread of pathogens that can impact public or animal health. Further, our observations highlight the need for fine-scale genetic approaches to characterize the distribution of diversity for species of *Trichinella.*