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TRICHINELLOSIS – ETIOPATHOGENETIC AND EPIDEMIOLOGICAL INVESTIGATIONS

SUMMARY OF THE PH.D. THESIS

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Introduction

Trichinellosis, a cosmopolit parasitic zoonosis, determined by nematodes from *Trichinella* genus, with intestinal development in adult stage and muscular in larval stage, in the same host, produce clinical signs in humans, following the ingestion of contaminated meat, manifested through digestive disorders, diffuse pain, allergies, fever, with a potential fatal evolution, and an asymptomatic evolution in more than 300 potential host of animal species.

The *Trichinella spp.* infection in domestic animals (mostly pigs), was documented in 43 countries (21.9%) and in 66 countries (33.3%) was reported in wild animals (Pozio, 2007b).

The biology, biochemistry and molecular biology research, allowed the *Trichinella* genus to be divided in 8 species and 4 different genotypes (Murrell et al., 2000; La Rosa et al., 2001; Pozio et al., 2002).

In Romania, for the moment, two of the species are present: usually *T. spiralis* in domestic animals and *T. britovi* in wild animals (Blaga et al., 2009).

Romania has the most cases of *Trichinella* infection in animals and in humans, in the world. During 1980-2004, in Romania were recorded 28,293 human cases which means 51.1 annual cases in 1 million inhabitants (Blaga et al., 2007).

The principal contamination source for humans is represented by raw meat consumption from pigs and wild boars. Pretty rare other infestation sources are documented, such as horse meat.

Thesis structure

The thesis was divided into two parts. The Bibliographic research part was composed from 5 chapters and the Personal research part was composed from 3 main chapters. In the Personal research part through experimental and epidemiological studies the aim was to clarify some aspects regarding the ethiopatogenesis, the immunity and the diagnosis of this parasitic disease.
1. BIOCHEMICAL, HEMATHOLOGICAL AND IMMUNOLOGICAL ASPECTS IN TRICHINELLA BRITOVI AND TRICHINELLA SPIRALIS EXPERIMENTAL INFECTION IN PIGS

This chapter was divided in three main parts:

- *Trichinella britovi* experimental infection in pig;
- *Trichinella spiralis* experimental infection in pig;
- The comparison of the changes occurring in host after the experimental infection with *T. britovi* and *T. spiralis*.

The aim of this study was to experimentally infect pigs with the two different species of *Trichinella*, and to observe the hematologic, biochemical and serological changes occurring.

The principle of the research was the correlation of the changes with the three phases of the biological cycle: adults in the gut, migration of the new born larvae and encysted muscular larvae.

In the first experiment, were 3 groups of animals: one was infected with 1500 *T. britovi* larvae/kg, one group was infected with 500 *T. britovi* larvae/kg and a control group. The second experiment was done with two groups of pigs: one infected with 1500 *T. spiralis* larvae/kg and one control group.

For evaluating the impact that the two species have on the host organism, the following hematological, biochemical and immunological parameters were measured: granulocyte, hemeties, haemoglobin, hematocrit values, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, platelet number, total platelet volume, mean platelet volume, platelet distribution width, ALP, CK, LDH, ALT, AST, De Rittis index, K+, serum creatinine, blood cholesterol, blood glucose concentration, phagocytes stimulation test, blastic transformation test, ELISA.

In *T. britovi* experimental infection in pigs, performed in the Parasitology and Parasitological Diseases Department in the Faculty of Veterinary Medicine in Cluj-Napoca, after the paraclinic investigations, we came to the following results:
The weight gain in the three groups of animals was inversely proportional with the infected dose, and the differences between groups were statistically significant (p<0.05);

The AST activity increased, 3 days p.i., in the first group (71.4 U/l) and 10 days p.i. in the second group (61.2 U/l), moment connected with intestinal and migration phase of the biological cycle of the parasite;

De Rittis index increased beyond the reference value, 1, 3 days p.i. in group 1 (1.32) and 10 days p.i. in group 2 (1.47); The LDH activity increased 10 days p.i. (intestinal and migration phase) in group 1 (921 U/l) and in group 2 (1004 U/l);

The creatinine values remained in the normal range, but comparative with control, the seric concentration increased in group 1, 24 days p.i. (1.08 mg/dl) and 75 days p.i. (1.16 mg/dl), which corresponds with the muscular phase of infection; An increase in the leukocyte number, was more obvious 17,24,38 days p.i.; The eosinophyls percentage (10.2%) increased beginning with the 17th day p.i.;

The CPK values, hematocrit, hemoglobin, red cell number, remained in the normal range during the experiment;

The antibodies titre, determined by ELISA, became obvious 17 days p.i. in the 1500 larvae group and 24 days p.i. in the 500 larvae group;

In *T. spiralis* experimental infection in pigs, performed in the Parasitology and Parasitological Diseases Department in the Faculty of Veterinary Medicine in Cluj-Napoca, after the paraclinic investigations, we came to the following results:

The weight gain in the infected group (6.6±0.8 kg) was significantly decreased (p=0.05) comparative with the uninfected control (8.8±1.3 kg); The leukocyte number in the infected group, 14, 30 and 60 days p.i. was increased comparative with control (23.38 \(10^9/l\), 22.93 \(10^9/l\)), but the values remained in the normal range;

The lymphocyte number in the infected group, 14, 30 and 60 days p.i. (6.99 \(10^9/l\), 6.80 \(10^9/l\) and 5.28 \(10^9/l\)) was elevated comparative with control, but the values were in normal range. The granulocyte number (16.55 \(10^9/l\)), 4 days p.i., was significantly (p=0.03) increased in infected group comparative with control; A slight eosinophilic (4.5%) appeared 30 days p.i.
CK, 30 days p.i., in the muscular phase, had an increased activity (253.3 U/l) in the infected group, comparative with control (196.8 U/l). ALT, 60 days p.i., activity was significantly (p<0.05) increased (92.17 U/l) in the infected group comparative with control (60.39 U/l); AST, 4 days p.i., had a decreased activity (43.73 U/l) 4 days p.i. in the *Trichinella* group, comparative with control (60.7 U/l); De Rittis index remained under the reference value 1, during the entire experiment, meaning that the larvae didn’t influence the liver activity;

The blood glucose, in the infected group (169.6 mg/dl), 30 days p.i., increased significantly (p<0.05), comparative with control (109.3 mg/dl). Values over the normal range in pig (66.4-116.1 mg/dl) were observed 4 and 14 days p.i. in both groups;

In phagocytes stimulation test, a slight increase of the phagocytes activity, 14 days p.i. (migration phase), but the differences were not significant (p>0.05).

A significant increase in the lymphocyte activity was registered 30 (p=0.003) and 60 (p=0.05) days p.i., comparative with control;

IgG antibodies became detectable by ELISA, 14 days p.i., with Pasteur kit, and 30 days p.i. with both kits. The ELISA Pasteur kit proved a higher sensitivity;

The most infested muscular groups were the diaphragm and tongue;

analyzing the results obtained during the *T. spiralis* infection in pigs it can be concluded that the negative impact that the parasite exhibits on the host is minimal and that the host and the parasite are in a certain balanced state.

2. EPIDEMIOLOGICAL STUDIES REGARDING *TRICHINELLA SPP.* INFESTATION IN SOME ANIMAL SPECIES FROM ROMANIAN COUNTIES

The epidemiological study was made during 2004-2011. Because in the trichinellosis epidemiology there are two major cycles: the sylvatic cycle and the domestic cycle (Blaga, 2009a), the present study was divided in three main chapters which include the two cycles and the animals species which represent the link between them: sylvatic fauna, transition fauna and domestic fauna.
**Sylvatic fauna**

A total of 116 mammals and birds from 8 different counties were examined. The muscular samples were collected from the election zones in each different species of animal and were examined with the artificial digestion method. In the positive cases, the larvae were collected and conserved in absolute alcohol, later, the DNA was extracted and the multiplex PCR was done (Pozio and La Rosa, 2003).

From the 116 animals examined, 21 (18.1%) were positive to the *Trichinella* infection. None of the 51 birds examined presented in muscles *Trichinella* larvae. From the 21 positive samples, 7 were infested with *T. spiralis* (33%) and 14 (66%) with *T. britovi*.

**Transition fauna**

During the November 2009 and June 2011, 186 specimens of rodents and small carnivores from 25 different species, from 12 different counties, were brought to the Parasitology and Parasitological Diseases Laboratory in frozen state. 17 of them belonged to Rodentia order and 8 to Carnivora order. All the 22 examined carnivores belonged to the Mustelidae family.

The methodology used for detecting the *Trichinella* larvae was the artificial digestion, the magnetic stirrer method (Dupouy-Camet și Murrell, 2007). The recovered larvae from the positive samples, were subjected to DNA extraction, multiplex PCR method and migration of the PCR products.

*Trichinella* larvae were recovered in 5 out of 22 examined mustelides (22.7%). From the 8 species, belonging to Mustelidae family, the infection was detected in 3 of them: *M. erminea* (3/3), *M. lutreola* (1/3) și *M. foina* (1/3). The PCR analyses revealed two different *Trichinella* species: *T. britovi* in two ermines (*Mustela erminea*) and a Stone marten (*Martes foina*) and *T. spiralis* in a European mink (*M. lutreola*) and a ermine.

This is the first mustelid epidemiological study, of Romania, that aimed the identification of *Trichinella* species through molecular biology tools being the first report of the presence of *Trichinella* genus in *Mustela erminea* and *Martes foina* from România, and the first report of the presence of *T. spiralis* in Mustela family in Europe.
From the 164 analyzed rodents only 3 (1.8%) were found infected. The 3 specimens belonged to two different species: 1 *Rattus rattus* and 2 *Rattus norvegicus*. All three specimens were collected from Cluj-Napoca and were infected with *T. spiralis*.

**Domestic fauna**

A number of serum samples from 246 cats, with a healthy clinical status, were examined in this study. These were from different rural or urban areas from Crisana, Oltenia and Transilvania regions, from: Alba, Bistrița-Năsăud, Brașov, Cluj, Dolj, Harghita, Mureș and Sălaj County. The age of the examined cats was between one month and 17 years.

The serum samples (246) were examined by ELISA (*Enzyme Linked Immuno Sorbent Assay*) in order to identify the IgG anti-*Trichinella* antibodies. A swine Bio-Rad ELISA kit was used. The kit conjugate was replaced with a peroxidase antilat monoclonal antibodies. The positive and the negative control were obtained by experimental infection of a 2 month old cat and a second kitten was taken as a negative control. The frequency, serum prevalence and confidence interval (IC 95%) of *Trichinella* infection in cats, were calculated with Hi test from EpiInfo 3.5.1. program.

From the total of 246 analyzed samples, 19 (7.70%) were found to be positive. The highest serum prevalence was found in old cats (13.3%, 2/15), and the lowest in young cats (2.6%, 1/39). Two main periods were critical for *Trichinella* infection: at 2.5 years (40%) and at 13 years (33%). The highest serum prevalence was observed in cats from urban areas (8.2%), from the north-western (14.2%) part of Romania. The lowest serum prevalence was observed in samples from south-eastern (1%) part of Romania. The differences between regions were statistical relevant (*p*<0.0022). The county with most cases was Cluj (16.5%) followed by Harghita county (10.3%). The differences between counties were relevant statistically speaking (*p*=0.0095).

This is the first seroepidemiological research regarding *Trichinella* infection in cats, from Romania, and one of a few worldwide.
3. EXPERIMENTAL RESEARCH REGARDING THE ANTITUMORAL POTENTIAL OF DIFFERENT SPECIES OF TRICHINELLA

The study was performed in March-July 2010, on white females Wistar rats, which were infected with different doses of *T. spiralis* and *T. britovi*. 3 weeks p.i. the rats were s.c. grafted with Walker 256 solid tumor. Three month later, after clinical examination, the rats were sacrificed; the tumors were collected and weighted. Mycroscopical sections were performed and these were stained with Haematoxilin-eosin and Tricrom-Masson method. The total body weight at the beginning and in the end of the experiment was similar in all animals. No significant statistical differences were observed in total body weight between the groups. The rats in the control group presented large tumors in the back zone and had cahectic look. the tumors in the *T. spiralis* group were small weighing 0.05-0.93 grams; only one tumor was over one gram; the tumors in the *T. britovi* group were weighing 0.04-41.3 grams; one of the animals presented in the back a metastatic tumor (41 grams).

The microscopic aspects of the tumors collected from controls revealed: tumor enclosed by a thin layer of conjunctive tissue, epithelial tumor cells without clear intercellular limits, nuclear and cellular pleomorphism, vascular invasion, basophilic cytoplasm, cells organized in nests separated by a thin layer of conjunctive tissue, pleomorphic cells, hyperchromatic with vesicular nucleus, large nuclei, sometimes more than one, infiltration of inflammatory cells, specially eosinophils and lymphocytes.

The microscopic aspects of the tumors collected from *Trichinella* infected group revealed: thick conjunctive capsule, dystrophic calcification of the tumor tissue.

In conclusion *Trichinella* infection provides a certain protection against the Walker 256 carcinosarcoma development. This is the first research worldwide that proves that *T. britovi* infestation provides an antitumoral protection.