

Biochemical, hematological and serological changes in experimental infestation with *Trichinella britovi* in pig

Modificări biochimice, hematologice și serologice la infestația experimentală cu *Trichinella britovi* la porc

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ABSTRACT

Several biochemical and hematological changes are currently observed during *Trichinella* infection in humans and animals. The aim of the present work was to detect biochemical, hematological and serological changes occurring during *Trichinella britovi* infection in pig. 5 pigs were infested with 15.000 larvae, respectively 5000 larvae. Blood samples were recovered individually for up to 80 days post-infection (p.i.). Increased values were revealed for ASAT, ALAT, LDH, CPK and eosinophils. No changes were identified for, PA, creatinine, hematies, hematocrite, hemoglobin. Serum antibodies identified by ELISA ES (Pourquier Institute) were revealed from day 10 p.i., for the group infected with 15.000 larvae (3/5 pigs reacted positively). By day 26 p.i. the entire group presented higher values than the cut-off. Furthermore, the pigs infested with 5.000 larvae were detected positive for antibodies anti-*Trichinella* beginning with day 40 post infection. Work founded by the Romanian Education Ministry through the "CEEX" project (contract 99-2006-2008).

Key Words: *Trichinella britovi*, biochemical, serological, experimental, infestation, ELISA.

Introduction

Today, eight species and three additional genotypes are recognized in the genus *Trichinella* (Pozio and Murrell, 2006). Trichinellosis, one of the widespread helminthic zoonoses, is still endemic in most countries of the European Union. In the past few years, advanced biotechnology has been used to re-examine the taxonomy, epidemiology and life cycles of etiological agents, providing additional information on the main factors contributing to the maintenance of these parasites in Nature. The old concept that pigs and rats are the main hosts of *Trichinella spiralis*, as still reported in many books, has been re-evaluated thoroughly (Pozio E., 1998).

Trichinella infection is common in animals and can spread from its reservoir in wild animals to synanthropic animals, domestic

animals and people. Different transmission patterns have been documented (Campbell, WC, 1988). They range from those in which humans do not play any role to those in which improper human behaviour is the only cause of transmission (Pozio, E., 1998; Casulli and col, 2001) The infection pressure of the parasite biomass present in sylvatic animals and human malpractice in animal husbandry together can easily favour the transmission of *Trichinella* from the wild to the domestic habitat (Pozio E., 2001). The growing

importance of sylvatic species in the persistence and re-emergence of trichinellosis in many regions was emphasized at the 10th International Conference on Trichinellosis (Dupouy-Camet and col., 2001) Few cases of finding of *Trichinella britovi* in domestic pigs are known (Serrano, F.J.,

1998). *Trichinella britovi* is known as the parasite that affects especially wild animals.

The aim of the present work was to detect biochemical, hematological and serological changes occurring during *Trichinella britovi* infection in pig.

Materials and methods

In this study we used *Trichinella* infected fox meat and 15 two month old pigs. The infected meat was examined and diagnosed with *Trichinella britovi* infection in Paris, AFSA Laboratory.

The infected meat presented an average of 57 LPG (larvae per gram). We recovered the larvae using the artificial digestion method.

The 15 pigs were divided into 3 groups:

- group A – 5 pigs – infected with 1500 larvae/kg
- group B – 5 pigs – infected with 500 larvae/kg
- group C – 5 pigs – control group

The infestation was produced by oral administration of the larvae suspension.

All the investigations that we made, were correlated with different stages of the biological life cycle of the parasite. We used as benchmark the study of Prof. Dr. Eronim Şuteu (Şuteu E., V. Cozma, 2004) which sustains that 7-12 days p.i. the new born larvae enter the lymph, reach the circulation and spread all over the body, after 8-12

- before infestation
- 3 days post-infestation
- 10 days p.i.
- once a week for 5 weeks
- once a month for 2 month

} 10 blood samples for each pig.

40 days post-inoculation we proceed to muscular biopsy to determine if the parasite is present in the muscle samples using the artificial digestion. In digestion, the muscle is treated with an artificial “stomach fluid” consisting of hydrochloride and pepsin, to free larvae from their capsules.

ELISA was used to detect the rise in antibody levels during infection. We used a POURQUIER Institute ELISA diagnostic kit and a SAFE PATH Laboratories diagnostic kit. The microwells contained E/S *Trichinella spp.* antigens.

Results and discussions

During our study we revealed increased values for ASAT, ALAT, LDH, CPK and eosinophils. No changes were identified for, PA, creatinine, hematies, hematocrite, hemoglobin.

days p.i. the larvae enter the muscle fiber. Angiogenesis around the nurse cell starts at approximately day 12 post-infection. It is preceded by a hypoxic event, which triggers the up-regulation of vascular endothelial growth factor (VEGF). As the parasite and the nurse cell grow, hypoxia and the need for nutrients increase: neovascularization continue (Anu Năreaho, 2006). 18-20 days p.i. larvae begin to coil inside the nurse cell. Their growth is rapid from day three to a couple of weeks after entry into the muscle (Despommier et al., 1991). In addition, the parasite stimulates the nurse cell to synthesize collagen IV and VI to form a capsule 21-45 days p.i. 5-6 month after the infestation, the nurse cell starts to calcificate and continues up to 15 or 16 months.

It is known that 7-10 days after the infestation, faeces contain infesting larvae, so we took and burned them up to 10 days post inoculation, to prevent reinfestation.

Blood samples were recovered individually for up to 80 days post-infection (p.i.). We performed hematological, serological and biochemical blood profile.

We pursue the dynamic evolution of ASAT, ALAT, LDH, CPK, PAL, creatinine, hematies, hematocrite, hemoglobin, leukocyte formula, number of leukocyte. We collected the blood samples as shown bellow:

Alanine transaminase or ALAT is a transaminase enzyme. Significantly elevated levels of ALAT often suggest the existence of other medical problems such as viral hepatitis, congestive heart failure, liver damage, biliary duct problems, infectious mononucleosis, or **myopathy**. Fluctuation of ALAT levels is normal over the course of the day, and ALT levels can also increase in response to strenuous physical exercise. When elevated ALAT levels are found in the blood, the possible underlying causes can be further narrowed down by measuring other enzymes. Myopathy-related ALAT levels can be ruled out by measuring creatine kinase enzymes (Gyboney, P.T., 2005). In swine the ALAT physiological values are 15-75 U/l (Kadar, L., 2002). 80 days p.i. we noticed an increase in

ALAT values in both infected groups (group A : 86,6 U/l; group B: 84,6 U/l).

Aspartate transaminase (AST) also called serum glutamic oxaloacetic transaminase (SGOT) or aspartate aminotransferase (ASAT/AAT) is similar to alanine transaminase (ALT) in that it is another enzyme associated with liver parenchymal cells. It is raised in acute liver damage. It is also present in red blood cells and cardiac muscle, skeletal muscle, and kidney and brain tissue, and may be elevated due to damage to those sources as well. ASAT was defined as a biochemical marker for the diagnosis of acute

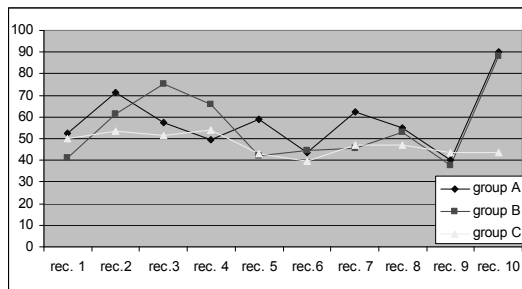


Fig. 1. ASAT

Lactate dehydrogenase (LDH) is an enzyme present in a wide variety of organisms, including plants and animals. In medicine, LDH is often used as a marker of tissue breakdown as LDH is abundant in red blood cells and can function as a marker for hemolysis. A blood sample that has been handled incorrectly can show false-positively high levels of LDH due to erythrocyte damage (Butt, A.A., and col., 2002). Values of 40-640 U/l, are considered normal in swine.

Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phospho-creatine kinase or sometimes wrongfully also creatinine kinase, is an enzyme expressed by various tissues and cell

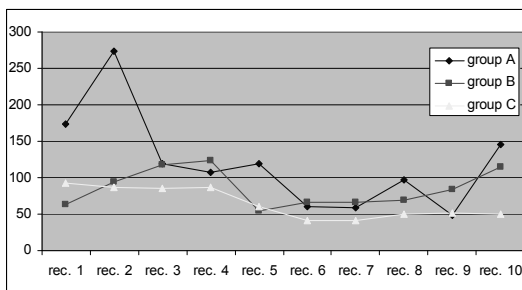


Fig. 3. CPK

In human trichinellosis some authors sustain the fact that all the muscle enzymes increase in serum: CPK, LDH, aldolase and only occasionally ASAT (Capo and Depommier,

myocardial infarction in 1954 (Gaze, D.C., 2007). In swine, the normal values of seric ASAT are 10-100 U/l. 80 days post-infection we registered a raise in group A (90 U/l) and group B (88 U/l) ASAT values, comparative with our control group (C), but we must say that the values were still normal.

In our study, ASAT and ALAT values raised 80 days p.i., period that corresponds with the end of the encapsulation. The evolution of these two enzymes is represented in the following graphics:

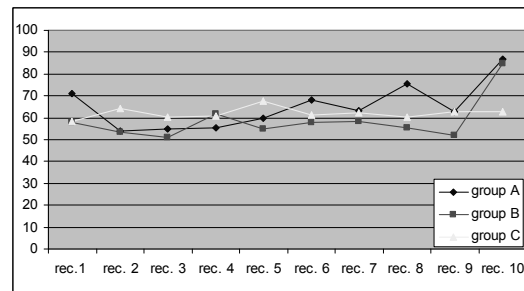


Fig. 2. ALAT

types. (Goldblatt H., 1969). Elevation of CPK is an indication of damage to muscle. It is therefore indicative of injury, rhabdomyolysis, myocardial infarction, muscular dystrophy, myositis, myocarditis, malignant hyperthermia, and neuroleptic malignant syndrome. (Wallimann, T., Hemmer W., 1994). Normal values of seric CPK, in swine, are 2,5-500 U/l. We must mention the fact that the LDH values increased when we performed muscle biopsy (1824,4 U/l – group A, 2099,4 U/l – group B). One week after the procedure, LDH values returned normal. CPK values increased 3 days after inoculation of the larvae in the first group: 274 U/l. The following graphics are relevant for our findings:

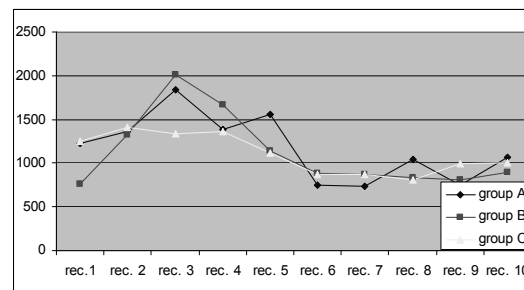
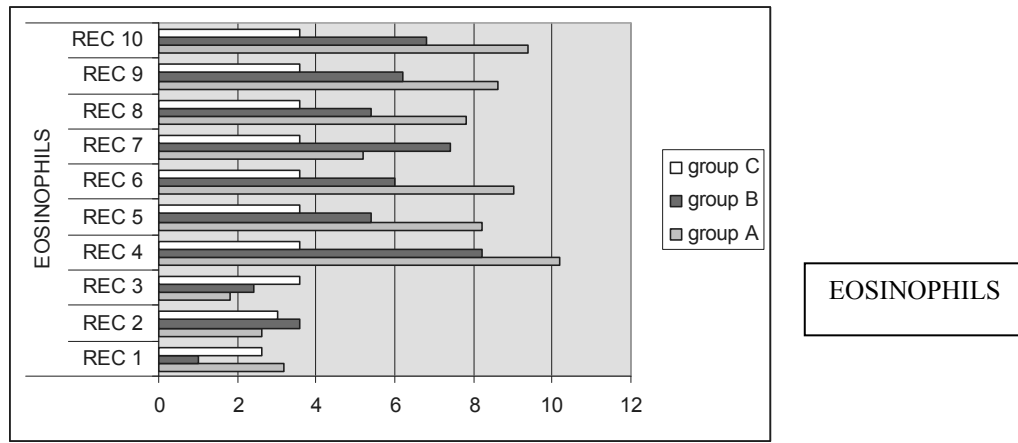


Fig. 4. LDH

1996). Laboratory Examinations revealed blood eosinophilia beginning with day 17 p.i.: group A 10,2%, group B 8,4%.



Eosinophilia is present, with few exceptions, in most cases of human trichinellosis, inasmuch as it is the earliest and most important host response. Even in human asymptomatic cases, increases in eosinophilia of up to 15% have been observed. Eosinophilia appears at an early stage of infection between the second and fifth weeks of infection (Dupouy-Camet J., 2002). No changes were

identified for, PA, creatinine, hematics, hematocrite, hemoglobin.

In terms of **dynamics of antibodies**, analyzed by ELISA, we found 3 positive samples in group A, 10 days p.i., all the samples in A group, became positive 26 days p.i.. 33 days p.i. 4 of 5 samples in B group became positive and 40 days after the inoculation, all the samples were positive.

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
O.D.	0,091	0,102	0,110	0,109	0,087	0,136	0,099	0,109	0,087	0,088	0,292	0,136	0,122	0,154	0,093	0,077	0,090	0,095	0,090	0,087	0,130	0,104	0,115	0,082	0,098	0,247	0,134	0,124	0,114	0,115
Inter.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Larvae /kg	1500	1500	1500	1500	1500	500	500	500	500	500	0	0	0	0	0	1500	1500	1500	1500	1500	500	500	500	500	500	0	0	0	0	0
Days	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. a.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.	3 d. p.i.

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
O.D.	1,252	0,274	0,800	1,249	0,468	0,105	0,077	0,110	0,098	0,111	0,089	0,080	0,085	0,075	0,080	1,420	0,612	1,118	1,414	0,828	0,096	0,102	0,131	0,141	0,088	0,080	0,090	0,080	0,088	0,075
Inter.	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-
Larvae /kg	1500	1500	1500	1500	1500	500	500	500	500	500	0	0	0	0	0	1500	1500	1500	1500	1500	500	500	500	500	500	0	0	0	0	0
Days	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	10 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.	17 d. p.i.

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D.o.	1,578	1,702	1,733	1,784	1,597	0,159	0,311	0,564	0,551	0,180	0,106	0,077	0,076	0,078	0,088	2,003	0,065	2,104	2,123	1,812	1,638	1,249	1,785	1,629	0,709	0,101	0,073	0,075	0,081	0,081
Inter.	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Larvae /kg	1500	1500	1500	1500	1500	500	500	500	500	500	0	0	0	0	0	1500	1500	1500	1500	1500	500	500	500	500	500	0	0	0	0	0
Days	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	26 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.	33 d. p.i.

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
O.D.	1,925	2,013	2,220	2,160	1,990	1,925	1,931	2,078	2,033	1,464	0,124	0,287	0,383	0,089	0,085
Inter.	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Larvae /kg	1500	1500	1500	1500	1500	500	500	500	500	500	0	0	0	0	0
Days	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.	40 d. p.i.

Legend:
O.d.- Optical density
a.i. – before infestation
p.i. – post infestation

Other authors mention earlier appearance of antibodies: beginning with day 6, when they used ELISA with crude antigen, but when they used ELISA with ES antigen, like us, positive titres appeared later, between days 16 and 20 p.i (Bolas-Fernandez F. and col., 1993).

Conclusions:

ASAT (90 U/l) and **ALAT** (86,6 U/l) values raised 80 days p.i., period that corresponds with the end of the encapsulation.

LDH values increased when we performed muscle biopsy (1824,4 U/l – group A, 2009,4 U/l – group B). One week after the procedure, LDH values returned normal.

Blood **eosinophilia** (10,2%) appeared 17 days after the infestation.

CPK values increased 3 days after inoculation of the larvae in the first group: 274 U/l.

Antibody titre, analyzed by ELISA, became relevant in group A, 10 days p.i., in group B, 33 days p.i.

No changes were identified for, PA, creatinine, hematies, hematocrite, hemoglobin.

REZUMAT

În timpul infestației cu *Trichinella*, la om și animale, se observă diferite schimbări la nivel hematologic și biochimic. Scopul studiului de față a fost detectarea schimbărilor biochimice, hematologice și serologice în timpul infestației experimentale cu *Trichinella britovi* la porc. S-au folosit 15 porci de 2 luni care s-au împărțit în 3 loturi: 5 au fost infestați cu 15.000 de larve; 5 au primit 5000 de larve; 5 au folosit drept martori. S-au făcut 10 recoltări de probe la intervale relevante pentru ciclul biologic al parazitului. S-a observat creșterea valorilor ASAT, ALAT, CPK, LDH și a eozinofilelor. Nu s-au înregistrat modificări ale valorilor PA, creatininei, hematiilor, hematocritului, hemoglobinei. Anticorpii serici au fost detectați prin metoda ELISA începând cu ziua 10 p.i., la grupul A (3 din 5 probe), ziua 33 p.i. la grupul B (4 din 5 probe). Toate probele au devenit pozitive 40 de zile p.i.

Cuvinte cheie: *trichinella britovi*, biochimic, serologic, infestatie, experimenta, ELISA.

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