

**National Center of Infectious and  
Parasitic Diseases  
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**To the President  
of the Scientific Jury, chosen  
by the Director of NCIPD  
with Order № 452/29.10.2019**

## Review of Doctoral Thesis

**Of Assoc. Prof. Dr. Borislava Georgieva Chakarova, PhD**  
Trakia University – Stara Zagora, Faculty of Medicine  
Department “Parasitology and Microbiology”

***Concerning dissertation thesis for the award of educational and scientific degree  
“DOCTOR”***

**Scientific field:** 4. Nature science, mathematics and information technologies

**Professional field:** 4.3. Biological Sciences

**PhD program** “Parasitology and Helminthology”

**Author:** Eleonora Marinova Kaneva

**Educational form:** Self-training education form

**National Center of Infectious and Parasitic Diseases, Sofia, Department  
”Parasitology and Tropical Medicine”**

**Thesis topic:** “Development and application of serological methods for the  
diagnosis of Toxocariasis and evaluation of their significance  
as markers for the disease stage in humans”

**Supervisor:** Assoc. Prof. Dr. Iskra Georgieva Rainova, DM, NCIPD – Sofia,  
Department ”Parasitology and Tropical Medicine”

I declare, that there is no conflict of interests between me and the author of the  
dissertation, according to par.1, p.3 and 5 of ADASRB.

### **1. Presentation of the doctoral student and the procedure**

Eleonora Kaneva graduated high school with advanced study of Biology and  
Chemistry in 1994 in Pleven. She graduated a Master degree “Molecular Biology” in  
Sofia University “St. Kliment Ohridsky” and has professional qualification “Molecular  
biologist” and specialty of “Virology”.

In 2006 she started work as a biologist in National Centre of Infectious and Parasitic Diseases, Sofia, department of Parasitology and Tropical Medicine” (DPTM), which it is currently implementing. Her professional obligations include the production of diagnostic tests and nutrient media, which are used in the medical parasitology, participation in the diagnostic process of the parasitic diseases, assistance in conduction courses in the program of AGE. In 2016 she is assigned as a PhD student in the program “Parasitology and Tropical Medicine”, self-training education form in Department of Parasitology and Tropical Medicine, National Centre of Infectious and Parasitic Diseases, Sofia. The thesis of the dissertation is: *“Development and application of serological methods for the diagnosis of Toxocariasis and evaluation of their significance as markers for the disease stage in humans”*, which is in accordance with her professional skills and qualifications. The procedure of preliminary presentation of the dissertation is conducted on 17.10.2019 to the department colleagues, who unanimously accept the readiness for defense in front of the Scientific jury.

## **2. Characteristic of the dissertation**

The dissertation is structured according to the Regulation of ADASRB, NCIPD, Sofia. It has 208 standard pages and ten chapters: introduction (1page), literature review (35 pages), aim and objectives (1page), individual research: materials and methods (13 pages), results from the individual research (79 pages), discussion of the results (32 pages), summary (2 pages), conclusion (2 pages), contributions (1.5), bibliography (24 pages), publications and participations in scientific forums, related to the dissertation (1 pages), summary of Bulgarian and English languages (5 pages). There are 36 tables and 54 figures. The bibliography includes 306 publications.

## **3. Relevance of the topic**

During the last couple of years the zoonosis diseases covered much more wide range of unsolved problems in the human pathology. The reasons for this large-scale process are diverse – changing territory of spread, new relationships in the epidemic processes, polymorphism of the clinical symptoms, dissatisfaction, searches and trays for diagnostic precision, varying treatment options. The doctoral student and her supervisor, Assoc. Prof. Dr. Iskra Rainova, research the numerous problems of toxocariasis in its medical aspects and decide adequately and on time to challenge themselves, as they perform series of examinations, analysis, discussions of the received results and forming conclusions, which they arrange in well structured dissertation paper.

#### 4. Understanding the problem

The deep understanding of the problem, which the PhD student has, is due to her wide research in the literature, which is presented in **the literature review**. There is useful information, like the historical data for toxocariasis, taxonomy, morphology, biological cycle and transmission of *Toxocara spp.*, such as the clinical symptoms in humans. The laboratory diagnostic methods, which are very well known and these ones, which are innovative, are very well described and have scientific interpretation, as well. This is the base of the developed and applied serologic diagnostic methods in the dissertation. An assessment and determination of their importance to the definition disease's stage of invasion, is also performed. 10 of 306 cited publications are from Bulgarian authors, written on Cyrillic, and 296 belong to Bulgarian and foreign authors, written in English language. 34% of the used literature is published after 2000 year.

The **aim** of the dissertation is well defined:

- development and evaluation of confirmatory serologic test for diagnostic of toxocariasis;
- determination of the diagnostic value of total IgE and specific IgA antibodies;
- establishing of toxocariasis's stage in humans, based on the received results.

The **objectives**, which are planned in alignment with the aim, are **5**. They are adequately formed and include consistent accomplishment of the main stages in the scientific project.

#### Materials and methods

Secretory-excretory antigen, received *in vitro* by cultivation of *Toxocara canis* larvae, is used. The examined serum samples are from enough number of patients (180) and 130 of them are with clinical and serologic data of toxocariasis. The control group consist 50 healthy blood donors.

Four trademark tests are used for determination of the specific immunoglobulins. They are manufactured in Germany and France: (1) Immunoenzyme method (ELISA) for detection of specific anti-*Toxocara* IgG antibodies; (2) Immunoenzyme method (ELISA) for determination of specific anti-*Toxocara* IgA antibodies; (3) Immunoenzyme method (ELISA) for determination the concentration of total IgE; (4) Western blot for defining of specific anti-*Toxocara* IgG antibodies. Series of statistical methods are selected properly and applied in order to receive results, which establish statistically significant

differences. The statistical correlations between the quantitative variables are determined by t-test, Anova,  $\chi^2$ , with level of statistical significance  $p < 0,05$ .

## **Results and Discussion**

The presented results, their discussion and comparison to these from the literature review, show very good analytical approach to the problem and adequate handling with the scientific literature. As a result are formed scientific aims. Laboratory ELISA tests for determination of anti-*Toxocara* IgG1, IgG2, IgG3 and IgG4 in human serum through definition of the optimal test parameters, are developed and standardized. The specificity and sensitivity of the ELISA laboratory tests are established. Among the patients with clinical symptoms of toxocariasis and proven anti-*Toxocara* IgG antibodies in the serum is established, that production of IgG1 is most common, such as the presence of all subclasses in the same time is very rare. Statistical significance between the production of anti-*Toxocara* IgG1 antibodies and the others subclasses (IgG2, IgG3 and IgG4) is found. Anti-*Toxocara* serum IgA antibodies are detect in  $\frac{1}{4}$  of the patients and their presence is commented as an indication for the early stage of the disease. It is found, that  $\frac{2}{3}$  of the patients has increased level of total serum IgE, compared to the accepted referent values for the Bulgarian population. The level of serum IgE is four times higher in infected children. It is highlighted, that the depletion of total serum IgE in the period between 6<sup>th</sup> and 9<sup>th</sup> month after the primary infection, gives the chance to determination of disease's activity and might give an indirect evaluation of the conducted treatment efficiency. The statistical data analysis shows, that the production of total serum IgE and anti-*Toxocara* IgG1 and IgG4 subclasses, has key role in the development of allergic immune response against the secretory/excretory antigens of *T. canis*. This leads to the state, that the specific IgA, IgG1, G2, G3 and G4 subclasses, such as total serum IgE, have important significance in immune response to the toxocariasis. The depletion of specific IgA and IgG2 in the period between 9<sup>th</sup> and 12<sup>th</sup> month after the diagnosis might be an indicator for chronification of toxocariasis invasion.

## **5. Contributions and significance of the dissertation to the science and practice**

The conclusions (10) are correctly formed in align with the aims and solved objectives and clearly resent the received results. The doctoral students determined 9 contributions, which differentiates as originals (6), scientific-practical character (2), practical character (1).

### **Contributions with original and scientific-practical character:**

- Standard laboratory ELISA tests, for determination the specific IgG subclasses, based on secretory/excretory antigen, received by *in vitro* cultivated *T. canis* larvae, are developed. Optimal test parameters are established and their diagnostic properties are assessed.
- Differences, in the specific IgG subclass immune response, are established by sex, age and clinical form of the disease.
- A correlation between separate IgG subclasses is found. Subclass IgG1 has significant role in the immune response to the toxocariasis and IgG2 running out in a year after the diagnosis.
- The diagnostic value of the specific IgA antibodies in toxocariasis is evaluated. Their detection in the serum give the opportunity to define whether it is early stage of the disease, due to their quick depletion in the course of the immune response.
- The examination of specific IgA antibodies and anti-*Toxocara* IgG2 subclass antibodies could distinguish acute from chronic stage of toxocariasis, because of their depletion in a year after the diagnosis.
- Relations between the production of specific IgG subclasses, specific IgA and total IgE antibodies are found. That gives important information about the humoral immune response in toxocariasis.

It is established, that increased values of total serum IgE in patient with toxocariasis have diagnostic meaning.

There is huge possibility for detection the stage of the disease and evaluation of the conducted treatment in the course of the complex examination the levels of the immune markers of toxocariasis.

**Contribution with practical character** is formulation of the diagnostic panel in toxocariasis and its implementation in the practice – tests for detection of specific IgG subclasses, immune markers of specific IgA and total IgE. The received information gives the opportunity to create a protocol for adequate treatment therapy.

### **6. Evaluation of the PhD student personal participation in the scientific part of the dissertation**

The period of data collecting and conducting the scientific activity is 10 years – between 2006 and 2015 year. This period couldn't be shorter, due to some objective reasons. The PhD student shows excellent personal skills after her assignment to self-training educational form in 2016 – ability for well organization, planning and methodology, confidence for accomplishing the dissertation before the deadline.

## 7. Assessment of the publications, related to the dissertation

The publications, related to the dissertation, are 4 and 2 of them are published in journals with impact factor: (1) Comptes rendus de l'Academie Bulgare des Sciences 2012, 65, 11, 1563-1568; IF - 0,210; (2) Parasite Immunology, 2015, 37, 505-509; IF - 2.143 (total IF 2.353). The participations in scientific forums are 4 and 1 is abroad. They present the results of the conducted researches. The PhD student is first author in 7 from 8 publications in scientific forums, which speak itself for her engagement and personal contribution to the dissertation.

The abstract has 70 papers and correctly present dissertation structure and main chapters. There are 19 tables and 30 figures, summary on Bulgarian and English language.

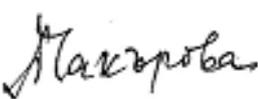
Some of my critical notes and recommendations are taken in consideration in the final version of the dissertation. The subsequent corrections are made creatively, according to her point of view.

## CONCLUSION

The dissertation shows, that the PhD student Eleonora Kaneva has theoretical knowledge and professional skills in the field of "Parasitology and Helminthology" and present qualities of conducting researches by herself. The applied diagnostic laboratory methods for toxocariasis, the received results, significant contributions in the dissertation, give me the reason to consider, that the presented scientific paper is in alignment with the requirements of ADASRB and the Regulations for its appliance in NCIPD, Sofia.

I evidently give my **POSITIVE** assessment of the conducted tests, received results and contributions. I suggest to the honored Scientific jury to award Eleonora Marinova Kaneva with educational and scientific degree "**DOCTOR**", scientific specialty "**Parasitology and Helminthology**", professional field 4.3. Biological science.

9.12.2019  
Stara Zagora

Reviewer: 

Assoc. Prof. Dr. Borislava Georgieva Chakarova, PhD