

EVALUATION REPORT

by **Professor Boyko B. Georgiev, DSc, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences**

on the dissertation thesis “Development and application of additional serological methods for the diagnosis of Toxocariasis and evaluation of their significance as markers for the disease stage in humans” for the award of the educational and scientific degree “Doctor” in the scientific specialty “Parasitology and Helminthology” presented by Eleonora Marinova Kaneva – a doctoral student on self-training education form, NCIPD.

The dissertation has up-to-date theme because it is focused on improvement of diagnostics methods of one of the most widespread parasitic diseases in Bulgaria – Toxocariasis. The aim of conducted research is to develop serological tests for diagnosis of toxocariasis and determination of the diagnostic value of common immunoglobulin E (IgE) and specific IgA antibodies for establishing the stage of the disease. The serological tests developed so far do not allow to identify whether the disease caused by the larval migration of cat’s and dog’s nematode *Toxocara* spp. in human body is in acute or chronic phase, an information important for evaluation of treatment efficiency. Therefore, I consider that presented research aims are well formulated and correspond to the to the main goal of the dissertation.

The research is based on abundant materials: 130 serum samples of patients with clinical and serological data of toxocariasis and 35 patients tested and analyzed second time, at later stage of the disease in a period of one year. Fifty samples of healthy people were used as a control group. Appropriate methods and research approach were applied in the dissertation – immunoenzyme method (ELISA) for determination of specific anti-*Toxocara* IgG and IgA antibodies; immunoenzyme method (ELISA) for defining the concentration of total serum IgE; Western blot for determination of specific anti-*Toxocara* IgG antibodies. Adequate statistical analyses, which provide assessment of received results, are applied. All experimental work is in accordance with the aim and the tasks of the research.

The results are presented clearly and systematically. The discussions are convincing and logically lead to the main conclusion of the research. They show the ability of the applicant to analyze and summarize scientific information.

There is no doubt, that presented research has a contributive caharacter. I consider as very important the following research contributions:

- Standardized laboratory ELISA tests are developed; these are based on *in vitro* cultivated larvae of *Toxocara canis* antigen and developed for determination of specific IgG subclasses antibodies (IgG1, IgG2, IgG3 и IgG4) and assessment of their diagnostic properties in *Toxocara*-infected patients.

- Differences in specific IgG subclass response of sex, age and clinical stage have been established, especially in regard to the levels of IgG1, IgG3 and IgG4 in adults and children, where the differences are statistically significant.

- The role of subclass IgG1 in the immune response of Toxocariasis is manifested.

- The depletion of IgG2 is established in a period of one year after the first diagnosis, which allows identification of the chronic stage of the infection.

- The fast negation of the specific IgA antibodies and anti-*Toxocara* IgG2 antibodies in a period of a year after the initial diagnosis of the disease is an original marker combination, which allows the reliable identification of the chronic Toxocariasis.

In my opinion, the determination of the significance and tracking the combination of immunological markers, which could determine the disease stage with high probability and treatment efficiency, are especially significant contribution with scientific and practical value. Important value has the implementation of the developed tests for determination of specific IgG subclasses. They will improve *Toxocara* diagnostics and will support the proper treatment.

There are 4 scientific publications related to the dissertation's topic. Two of them are in journals indexed by Web of Science and one is indexed by Scopus. The fourth paper is a full-text report in a collection of scientific papers of a national scientific forum. The results of dissertation are disseminated with four reports at scientific forums in Bulgaria (3) and abroad (1).

I do not have any critical comments on the dissertation.

In my opinion, the presented dissertation is in alignment with the requirements of the Regulations for the Application of the Law on the Development of the Academic Staff in the Republic of Bulgaria in his four versions, applied in the past 9 years. This refers to requirements for both professional areas 4.3. Biological science and 7.1. Medicine.

The dissertation has demonstrated that the doctoral student is formed as an excellent helminthologist – immunologist. She is well-acquainted with the literature and applied different methodological approaches. A great amount of parasitology material has been collected. The presented texts demonstrate logic, analytical and excellent handling with terminology. The conclusions and scientific contributions are clearly formulated and I agree with them. All this gives me reasons to support the award of the educational and scientific degree “Doctor” to Eleonora Marinova Kaneva.

14.12.2019

Signature:



(Prof. Boyko B. Georgiev, DSc)