

To the Director of the NCIPD

PROF. T. KANTARDJIEV, MD, DSc

REVIEW

By Acad. Vasil Grigorov Golemanski, DSc,

Institute of Biodiversity and Ecosystem Research (IBER) - BAS

Subject: Dissertation thesis for the award of the scientific degree

"Doctor of Science"

Topic: Helminthozoonoses (trichinellosis, toxocariasis and echinococcosis) in humans - epidemiological indicators, distribution, diagnosis and treatment"

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Professional field: 7.1 - Medicine

Scientific specialty "Parasitology and Helminthology"

1. General overview of the procedure: According to Order No. 504/16.12.2019 by the Director of the NCIPD, I have been elected as a member of the scientific jury in connection with the procedure for defense of the dissertation work entitled "Helminthozoonoses (trichinellosis, toxocariasis and echinococcosis) in humans) - epidemiological indicators, distribution, diagnosis and treatment" submitted for the degree "Doctor of Science" in the professional field 7.1 Medicine and the scientific specialty "Parasitology and Helminthology". The author of the thesis is Assoc. Prof. Iskra Georgieva Raynova from the Department of "Parasitology and Tropical Medicine" at the NCIPD.

2. Brief biographical information for the candidate: Assoc. Prof. Iskra Rainova graduated from the Medical University in Sofia in 1982. She joined the Department of "Parasitology and Tropical Medicine" (DPTM) at NCIPD in 1987, where she has been working. In her scientific career at the institute she has acquired two additional specialties - "Medical Parasitology" and "Clinical Immunology" and in 2006 defended a PhD thesis on the topic "Development and characterization of ELISA and Western blot with excretory-secretory antigens of *Toxocara canis* for diagnosis and study the spread of toxocariasis in our country". She is a longstanding scientists parasitologists in NCIPD, since 2010 holds the academic position of "Associate Professor", from 2011 to 2016 she was a head of the DPTM and from 2016 till now a Deputy Director of NCIPD and a chief coordinator of the expert council for Medical Parasitology at the Ministry of Health.

3. Relevance of the dissertation: The subject of study in the dissertation of Dr. I. Rainova are three of the widespread helminthozoonoses in our country: trichielloosis, toxocariasis and echinococcosis. Despite the changes in the public and cultural life in our country in recent decades, those zoonoses are still very relevant and important for our country. Particularly common are annual cases of trichinellosis and echinococcosis, in which Bulgaria is one of the foremost places in the European Union, despite the active prevention of anti-parasitic services in the country and information activity in the media. The objective data and analyzes of these diseases in the world and in our country, presented by Dr. Rainova in the introduction and the first chapters in the dissertation, are more than alarming and impose our country to take more fast and effective measures to limit the parasitoses cited. Moreover, as a result of the migration processes that have been developing in Europe in recent decades and of climate change in the world, it is possible in the future that the negative trends in the maintenance and even expansion of these dangerous helminthozoonoses in Bulgaria may continue, which is unacceptable for the 21st century. I believe that the proposed large-scale study of these parasitoses in Dr. I. Rainova's dissertation is relevant and practically significant and will be a serious fundamental basis for future activity for the reduction of parasitic diseases in our country.

4. Knowledge of the literature: The separated "literature review", developed in the thesis of around 70 p., makes a positive impression to the general parasitological culture and excellent knowledge of the problem by the author of the study both in our country and in the wider context - Europe and the world. Particularly relevant and in-depth is the analysis of the epidemiology, the clinic, the spread and the diagnosis of trichinellosis, echinococcosis and toxocariasis in our country for the last 18 years (2000 - 2017), which allows the author to make convincing scientifically sound conclusions and recommendations for the future activity of the medical parasitological science in Bulgaria in the control of these diseases. As a zoologist and parasitologist, I highly appreciate the section in the Literature Review on the characteristics of the species and genotypes of the causative agents of helminthoses, their biological cycles and epidemiological features, their geographical distribution in the world and in Bulgaria. This makes the dissertation very useful especially for young parasitologists and doctors in connection with their future activity in our country. With important practical significance are also analyzed issues concerning the risk factors for the existence and maintenance of surveyed parasitoses in Bulgaria, as well as examining the diagnostic (incl. modern molecular biological) methods for accurate diagnosis and treatment of these helminthoses. The relevance and precision of the literature review is also confirmed by the considerable number of bibliographic units analyzed - over 420 scientific articles, monographs, reviews, etc., cited precisely in the section "Bibliography" of the dissertation (pp. 216-240). A considerable part of literature sources have been published in the last 5-10 years, which makes them especially relevant and useful.

5. Assessment of materials and methods used: The importance of the dissertation is the fact that the research for the elaboration of the dissertation covers a long period of time

- 18 years (2000 - 2017), which gives a real enough picture of the dynamics of the investigated helminthoses in our country. The "Material and Methods" section is elaborated in detail in the dissertation and covers a large contingent of individuals from different regions of the country with a total of over 12,000 people. For the purpose of the research the author has used a wide range of classical parasitological methods, as well as more advanced methods for testing of antigens, serological and molecular biological methods, and others. Part of the methods used are detailed in the literature review of the dissertation. This section is addressed to some practical problems with regard to diagnosis, treatment and drugs used against studied helmintozoonoses, which makes the study especially useful for students, young parasitologists and network for parasitological laboratories in the country. It should also be noted that in the process of research and presentation of results in the dissertation, modern computer programs for statistical data processing were used, which is a guarantee for the accuracy and objectivity of the researches and conclusions made by the author.

6. Evaluation of the scientific contributions and importance of the dissertation: The main scientific contributions and achievements of Assoc. Prof. I. Rainova, presented in the presented dissertation, can be summarized in the following main points:

6.1. A detailed retrospective review and up-to-date analysis of the epidemiological picture in Bulgaria was carried out in relation to three widespread and socially significant helminthoses: cystic echinococcosis, trichinellosis and toxocariasis. A positive trend of decline in the cases of echinococcosis and trichinellosis in the country is established, but it is still too low for the study period of 18 years and these helminthoses remain in the focus of our parasitology offices and laboratories. Such a weak tendency to decline is also found in the mortality of these parasitoses. But the registration of more than 170 deaths in echinococcosis only in the last two decades is worrying for our country for the 21st century.

6.2. A fundamental and practically important result is the identification of the role of wild boars and domestic pigs in the epidemiology of trichinellosis in the country over the last 18 years. On the basis of these, a forecast of the expected trends in the epidemiology of the disease over the next 5 years was performed. Special attention from the medical parasitologists deserve and established facts that toxocariasis and echinococcosis affect more often female population in the country, and trichinellosis - men, primarily at a young age to about 40 years.

6.3. The current state of echinococcosis and trichinellosis in Bulgaria has been outlined on the basis of large-scale studies in the country. A high number of cases of echinococcosis was found in 3 districts in the country: Plovdiv, Burgas and Sliven, and trichinellosis - in Lovech and Sliven. In the future, these regions are subject to longer parasitological monitoring in order to reduce the incidence of these parasitoses.

6.4. The candidate elaborated and used new laboratory tests in this study - the ELISA IgG avidity test and the Western blot tests to evaluate the immune response in patients with clinical and serological data for trichinellosis and toxocariasis. They can be successfully used

for more accurate diagnosis and therapy of the parasitoses examined and in regional parasitology offices and laboratories.

6.5. A sero-epidemiological study of healthy individuals was performed to detect latent toxocariasis, resulting in the finding that this helminthosis occurs very often as a hidden invasion and is actually much more widespread in our country. There has been a sero-epidemiological study of healthy subjects to establish roundworms, resulting in it was found that this helminth infection occurs very often as a hidden invasion and in fact there are quite widespread in our country.

6.6. An analysis and evaluation of the financial costs of outpatient and clinical cases of trichinellosis in the country was carried out, which opens opportunities for the optimization and reduction of these costs for their treatment.

7. Publication activity of the candidate and its impact in the medical literature: Assoc. Prof. Dr. I. Rainova has significant personal contribution and active publishing activity on the problems of medical parasitology and especially of helminthozoonoses, the subject of the presented doctoral thesis. Her publishing activity is expressed in the authorship and co-authorship of 5 specialized chapters in Bulgarian monographs and educational manuals in the field of medical parasitology, 12 journal scientific articles in Bulgarian and international scientific journals in English with Impact factor, among which are the respected journals of "Parasitology Research", "Parasite Immunology", "American Journal of Tropical Medicine & Hygiene", and others. Part of the research of Dr. Rainova are published in 15 scientific articles in journals and collections of reports from scientific congresses and conferences in Bulgaria and abroad. She has participated with scientific reports, presentations and posters - more than 20 - and in many national and international scientific congresses, conferences and symposia in several countries (France, Italy, Romania, Turkey, Harvatan etc.). Her scientific articles and reports are cited in more than 20 foreign and Bulgarian parasitological publications, dissertations and others. The pointed facts and figures give me a reason to believe that Assoc. Prof. I. Rainova is an established and reputable Bulgarian parasitologist and the dissertation presented by her is a logical result of a long active research. In the course of her activity as an employee of the NCIPD, Dr. Rainova has actively participated in the development of 3 projects on the problems of parasitoses, financed by the National Science Fund.

8. Evaluation of the dissertation abstract: A detailed abstract of 78 pages is presented to the dissertation, which is in accordance with the structure and content of the dissertation, performed well the major contributions and achievements of the candidate and can be published for the purposes of defense.

CONCLUSION

The dissertation of Assoc. Prof. Dr. Iskra Rainova summarizes the results of a multi-year and in-depth comprehensive study of three very important and still widespread helminthozoonoses in our country: trichinellosis, toxocariasis and cystic echinococcosis. Due to

its relevance, significance and scope of the problems addressed in it, the work is a serious contribution not only to human medicine, but also to specialists in the field of veterinary medicine, healthcare and a number of local and national management structures interested in limiting and eventually eliminating these diseases in our country. In the dissertation work is given enough space on the methods used by the author in the process of this study. This makes the work especially useful not only for specialists in the parasitological network in Bulgaria, but also for students and young parasitologists who are focusing on science and practice in this field. The literary review, as well as the frequent references and citations in the work of a large number of Bulgarian and foreign scientists, who have worked on the three examined helminthoses - a total of over 420 bibliographic sources, a considerable part of which are already bibliographic rarity, deserves high praise. Some of the results in connection with the presented dissertation have already been published in reputable foreign and Bulgarian journals and have received positive reviews and evaluations from specialists. I believe that the proposed dissertation of Dr. I. Rainova is a serious achievement not only for her as a specialist parasitologist, but also for the NCIPD, in whose laboratories has conducted and completed this large-scale study. I suggest that the honorable leadership of the Institute to assist in the preparation and publication of the thesis as a separate monograph, which I am convinced it will present well among professionals and the public not only its author as a specialist parasitologist, but also the institute as the National Center of Infectious and Parasitic Diseases .

On the basis of the stated in the review and my many years of personal impressions of the doctoral candidate as an active and productive scientist - parasitologist, I firmly propose to the venerable Scientific jury to award the scientific degree "Doctor of Sciences" to associate professor Dr. Iskra Georgieva Rainova, MD, PhD.

Sofia, 10.01. 2020

Reviewer:



(Acad. V. Golemanski, DSc)