

To the chairmen of the scientific jury
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by the Director of NCIPD

REVIEW

By Prof. Rossitza Ivanova Kurdova-Mintcheva, MD, PhD,

Scientific Jury Member,

Scientific specialty "Parasitology and Helminthology"

National Center of Infectious and Parasitic Diseases, Sofia

On dissertation work for the award of scientific degree

"DOCTOR OF SCIENCE"

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

Author of the thesis Assoc. Prof. Iskra Georgieva Raynova, MD, PhD

**In higher education 7 "Healthcare and Sports"
professional field 7.1. Medicine**

Scientific specialty "Parasitology and Helminthology"

Analysis of the career profile of the candidate

Associate Professor Dr. Iskra Georgieva Rainova was born on 05.07.1956 in Sofia. After completing her medical education at MU Sofia in 1982, she initially worked as a district pediatrician at Regional Hospital in Dupnitsa (1982-1985) and a resident at the Fourth City Hospital in Sofia (1985-1987). In 1987 Assoc. Prof. I. Rainova joined the Department of Parasitology and Tropical Medicine at the National Center for Infectious and Parasitic Diseases (NCIPD), Sofia and consequently holds the posts resident physician at the Laboratory of Experimental and Applied Parasitology, Research fellow III-I degree, Head of Laboratory and Head of the Department of Parasitology and Tropical Medicine, and since 2016 she is deputy director of NCIPD. She is the Chief Coordinator of the Expert Council on Medical Parasitology at MoH. In 2006, she defended her PhD thesis on the topic: "Development and characterization of ELISA and Western blot with excretory-secretory antigen from *Toxocara canis* for diagnosis and study the distribution of toxocariasis in Bulgaria", and in 2010 acquired the academic position Associate Professor of Parasitology and Helminthology. She has two acquired specialties - Medical Parasitology (1992) and Clinical Immunology (1996).

According to the information provided, Assoc. Prof. I. Rainova has published a total of 87 articles - self-published and co-authored (70 in Bulgarian publications, 5 of them with IF, and 17 abroad, including 9 with IF), has participated in 6 collective monographs and manuals.

The total IF is 22. She has participated with posters and reports in 143 national and international scientific forums.

She is a member of the Bulgarian Parasitology Society, the Bulgarian Association of Medical Parasitologists and the Bulgarian Medical Association.

She is fluent in Russian and English languages - both written and spoken.

The relevance and importance of the dissertation

The dissertation work deals with a group of diseases – helminthozoonoses, of significant health and social importance. Studies have focused on three of them - trichinellosis, toxocariasis and echinococcosis, which are not only nationally targeted, but also of global interest for a number of reasons:

➤ Echinococcosis is an important public health problem with more than 1 million affected people worldwide. Regions of high endemicity include the Eastern Mediterranean, Northern Africa, Southern and Eastern Europe, South America, Central Asia, Siberia and Western China, where annual human morbidity can exceed 30 per 100,000 population (Integrating neglected tropical diseases into global health and development. WHO, 2017). Bulgaria refers to the echinococcosis affected countries as evidenced by annual analyzes of parasitic morbidity in Bulgaria and by a number of publications as well as from data from authoritative international organizations. Moreover, every year in our country officially are reported most cases of cystic echinococcosis in the European Union - 1807 diseased persons in 2011-2017 (ECDC, 2012-2017).

➤ Trichinellosis, which is most commonly epidemic, continues to be a problem for public health. Worldwide by trichinellosis are affected about 11 million people, each year are registered about 10,000 new cases (Lawley, 2013). In the European WHO region incidence in 1986-2009 ranged from 1.1 to 8.5 per 100 000 population (Murrell, Pozio, 2012). We have an annual outbreak epidemic outbreaks of trichinosis in humans. During 1990-2006 were registered 145 outbreaks with 4 108 officially registered patients with clinical manifestations for trichinellosis and with two deaths (Kurdova-Mintcheva et al., 2009).

➤ Toxocariasis is a neglected parasitic zoonosis with increasing public health impact that affects millions of people of the world. We have high (13%) seropositivity among patients, studied on clinical indications (Rainova, 2001; Rainova et al, 2006).

➤ Because of the health and social importance of these helminthoses, they are monitored by many international organizations. They are assigned as a neglected tropical diseases (NTDs) whose relevance to achieving the Global Goals sustainable development by 2030 is assessed by the WHO, which recommends their integration into global health and development programs. Echinococcosis is included in the WHO PTC program and is subject to monitoring, supervision and control. Trichinellosis is observed by International commission on trichinellosis), the European Food Safety Authority (EFSA), and the European Center for Disease Prevention and Control (ECDC).

➤ Echinococcosis and trichinellosis are subject to mandatory registration in Bulgaria by conducting epidemiological research and filling in an individual unified card for each case from the RHI. Although the numerous studies of these helminthoses in our country, all of the above require a permanent epidemiological surveillance, monitoring, updating and analysis of the main epidemiological parameters. This information is with key importance of

making adequate management decisions and conducting effective early diagnosis and treatment measures for and limiting the incidence and spread of helminthozoonoses.

All the aspects defined above prove the dissertation work as meaningful and relevant.

General characteristics of the dissertation

In the dissertation, the classical structure is consistent with the Law for the Development of the Academic Staff in the Republic of Bulgaria, and the Regulations for its implementation in NCIPD including the following heads:

Introduction - 1 page,

Literary Review - 64 pages,

Purpose and objectives - 1 page.

Materials and Methods -10 pages,

Results and discussion of own research -124 pages,

Conclusions - 3 pages,

Self-assessment of contributions to the thesis - 2 pages,

Bibliography - 24 pages,

Publications and participations with reports in scientific events related to the dissertation work - 3 pages,

Summary in Bulgarian - 4 pages,

Summary in English - 4 pages

The dissertation is written on 257 standard pages in Bulgarian. It is well balanced, and the most attention is paid to the Results and Discussion section of the own studies. It should be emphasized the rich visualization with appropriate selected and informative figures (59) and tables (34) that well represent the results obtained and the summaries performed.

Review of the dissertation work and evaluation results

Knowledge of the problem. Literature review. The material outlined in the literature review indicates that the doctoral student is well informed about the problem both globally and nationally, based on the analysis of 427 sources, 88 of which are in Cyrillic and 339 in Latin. The knowledge and coverage of the numerous studies by Bulgarian authors on helminthoses, as well as the citation of leading international organizations such as WHO, ECDC, EFSA and others, is very impressive. The review is dedicated to the specific topic of the dissertation, but in her pursuit of completeness, the student also included data that was not specifically for the subject of the study, such as the morphology and life cycle of the parasites, as it is more appropriate for monograph work.

Based on the literature review, the doctoral student formulates the purpose and tasks of the dissertation work well.

Purpose and tasks. The purpose of the dissertation is "to study the epidemiological, clinical and diagnostic features of helminth infection (trichinellosis, toxocariasis and echinococcosis) in the population of the Republic of Bulgaria for the period 2000-2017.

The five tasks formulated reflect the main directions in the development of Assoc. Prof. I. Rainova and specify the planned studies.

Materials and methods. The study covers considerable time period - 18 years (2000-2017). The large volume of data processed and analyzed from various sources is impressive: official reporting documentation and cards for the epidemiological study of echinococcosis

and trichinellosis prepared in Regional Health Inspectorates, parasitic disease analyzes prepared by NCIPD and the National Center for Public Health and Analyzes, reports from the National Statistical Institute (NSI) and the Health Insurance Fund and her own studies. Toxocariasis is not included in the mandatory registration list of the Ministry of Health (MoH) and is therefore summarized data for patients with clinical manifestations, healthy individuals and risk contingents examined in the Department of Parasitology and Tropical Medicine during the studied period. Appropriate modern diagnostic-parasitological and serological methods (IHA, ELISA and Western blot) have been applied, as well as epidemiological and statistical methods. Statistical processing of the data justifies the results and conclusions to be objective.

Evaluation of results, their analysis and interpretation

The results of the study and their discussion are presented together in three main sections, devoted respectively to trichinellosis, toxocariasis and cystic echinococcosis, the contents of which are consistent with the tasks assigned.

As a result of a complex epidemiological study for a period of 18 years (2000-2017), Assoc. Prof. I. Rainova makes a modern clinical and epidemiological characteristic of the most significant in Bulgaria helminthoses, analyzing the data of a large contingent of affected persons - 2,068 with trichinellosis, 8,157 with cystic echinococcosis and 2,087 with toxocariasis, which updates the information on these parasitoses in the country. The results obtained are a good basis for formulating future measures to improve epidemiological surveillance and control of these parasitoses at national and regional level.

Indisputable dignity is the characteristic of the main epidemiological parameters in the dynamics, which establishes a trend of a gradual decline in the cases of trichinellosis and echinococcosis and the average annual incidence - from 5.9 per 100,000 population in 2009 to 0.78 per 100,000 population in 2017 from trichinellosis and from 8.2 per 100,000 population in 2002 to 2.8 per 100,000 population in 2017 for echinococcosis, but these values remain high compared to other European countries. The doctoral student explains the decline in trichinosis mainly by increasing control in large pig farms after EU accession. Logical explanation for the downward dynamics of echinococcosis incidence the author finds in the successfully implemented National Program for the control of echinococcosis in humans and animals in Bulgaria (2004-2008). The author makes the forecast that this decline will continue in the coming years and after five years the incidence for trichinellosis will reach 0.5 per 100,000 population, and for echinococcosis - below 2.0 per 100,000 population. The data on differences in standardized and actual incidence of trichinellosis and their coincidence in echinococcosis deserve attention. Assoc. Prof. I. Rainova makes the important conclusion that despite the established reduction of the incidence of cystic echinococcosis and trichinellosis, Bulgaria continues to be the leading in this indicator among the EU member states. Original data on the seroprevalence of toxocariasis, with the high relative part (13.7%) of positive among persons with clinical signs clearly indicates the important role of this zoonosis in a number of clinical conditions (allergies, eosinophilic syndrome, ocular pathology, etc.), and the established 6.7%, seropositivity of the conducted sero-epidemiological study among healthy persons is an indicator for the presence of significant latent morbidity in our country. The key importance in controlling parasitoses is the elucidation and characterization of the main units of the epidemiological process - the source, the mechanisms of transmission and

the susceptible population to which the author logically refers. For this, I cannot ignore the data from the identified sources of trichinellosis outbreaks that clearly show a change in trends. Whereas in the previous period (1990-2006), part of which also covers 7 years of this study, most of the *Trichinella* outbreaks were caused by consumption of meat from domestic pigs (Kurdova et al, 2009), the overall indicator for the years 2000-2017 shows predominance of wild boars (in 43 outbreaks the source is wild bear, and in 38 - domestic pig) and although the differences are statistically insignificant, it is an indicator of higher transmission intensity in the environment and improved control in the rearing of domestic pigs. However, a certain concern is the fact that Assoc. Prof. Rainova found that a large number of sporadic cases (46 of 103) of trichinellosis, as well as some epidemic outbreaks (Sofia, 2000; Blagoevgrad and Sofia, 2002 - 70 patients; Plovdiv, 2004) occurred as a result of meat products consumption from a commercial network or catering service, which is an indicator of food safety control gaps. The profile data of the persons affected are important for the epidemiological surveillance and for prioritizing preventative measures. Depending on sex, men are more likely to suffer from trichinellosis, and women are more likely to have toxocariasis and echinococcosis. While trichinellosis and toxocariasis are more common in cities, echinococcosis is more prevalent in villages. The most common affliction of helminthozoonoses in young people and children is particularly worrying and should provoke special measures to be taken in these contingents, especially in the context of the demographic crisis in our country. Moreover, despite the decline in mortality and lethality in echinococcosis during the observation period, the doctoral student found that out of 175 deaths, 9 were children. The geographical distribution of helminthoses in our country is also a subject of deep analysis. The doctoral student finds that *Trichinella* outbreaks have been registered almost over the years all over the country - in 24 of the 28 districts, in 50 cities and 42 villages, and in some of them (Rila, Kyustendil; Obzor and Karnobat, Burgas region) this has happened repeatedly, the average morbidity ranges from 0.05 per 100,000 population in Ruse region to 8.1 per 100,000 population in Lovech region. Echinococcosis is registered everywhere, but traditionally the most affected is the population of Plovdiv, Sliven and Burgas regions, which again shows the need for complex, long-lasting and sustainable measures in these regions. The definition of the indicator Attack rate (AR) which characterizes the severity of trichinellosis outbreaks and in some cases reaches 100% is of particular contribution. An interesting fact is that the average rate of outbreaks caused by consumption of domestic pig meat is higher (39%) than that of wild boar sources (28%), however, the lack of statistical processing and data on the type of *Trichinella* species that caused some of the outbreaks makes it difficult to interpret these results reliably. It is also necessary to note the data from the clinical and laboratory studies of infected persons, which confirm the peculiarities of the clinical course of trichinellosis, toxocariasis and cystic echinococcosis.

It should be highly rated the analysis of the data for chemoprevention of cystic echinococcosis which is performed in only 26% of all operated patients. This information is crucial because it strongly indicates that the recommendations of the WHO and other international organizations are underestimated, leading to a significant number of recurrences of the disease in our country.

I would especially like to acknowledge and appreciate the analysis and the importance of determination for the first time in Bulgaria of the medical costs caused by trichinellosis after the introduction of the health insurance system. They are significant, amounting to a total of 333 941 leva for the period from 2005 to 2017 and are a clear signal for the need to strengthen organized preventive measures.

A considerable part of the dissertation is devoted to the evaluation of the applied diagnostic laboratory methods and to the development of new immunological tests for the diagnosis of trichinellosis, toxocariasis and echinococcosis.

According to data from the epidemiological cards for cases of trichinellosis and echinococcosis, the author indicates that in the Republic of Bulgaria primarily are used IHA and ELISA, but it makes an impression the low relative part (43%) of serologically examined persons with registered echinococcosis. Late anti-trichinella antibody positivity was detected in ELISA - average after a 40 days since the infection.

Particular attention should be paid to the development by the doctoral student of laboratory variants of Western blot with good specificity and sensitivity for the confirmatory diagnosis of trichinellosis, toxocariasis and echinococcosis, which could be used for reference diagnosis in NCIPD.

The development of standardized laboratory ELISA IgG avidity tests for the early diagnosis of trichinellosis and toxocariasis is beneficial, and allow to determine the timing of chronicity of the immune response in patients with clinical and serological data for these parasitoses.

Assoc. Prof. I. Rainova adds to the complex of diagnostic methods for toxocariasis the study of the level of eosinophilic cationic protein (ECP) secreted by activated eosinophils and concludes that it can be applied as a possible marker for establishing the stage of infection with *Toxocara sp.*

Of particular practical importance is the compilation of a diagnostic algorithm for toxocariasis as a result of a study of world experience and her own data.

The conclusions of the study as well as the development and the application of complementary confirmatory laboratory ELISA IgG avidity and Western blot provide an opportunity to improve diagnostics of trichinellosis and toxocariasis in our country.

Assessment of conclusions and contributions

The author formulates 17 conclusions which are outlined from the results obtained and emphasize the relevance of the studies and their theoretical and applied relevance. Due to the large volume of the study, I think that the conclusion on the role of eosinophilic cationic protein (ECP) was not reflected as a possible marker for the identification of the infection stage with *Toxocara sp.*

I accept the contributions from the self-assessment of Assoc. Prof. I. Rainova and consider that they reflect objectively her real achievements.

I appreciate the following contributions as more significant and original:

1. The contemporary epidemiological characteristics of trichinosis, toxocariasis and cystic echinococcosis in the population of the Republic of Bulgaria for the 18-year period (2000-2017) updates the information on the main epidemiological parameters and trends in these zoonoses in Bulgaria. The serological screening of healthy individuals and some risk groups expands and enriches the prevalence of toxocariasis in the country.

2. The developed laboratory ELISA IgG avidity test provides an opportunity to determine the timing of immune response chronicity in patients with clinical and serological data for trichinellosis and toxocariasis.

3. For the first time in Bulgaria, the medical costs caused by trichinellosis after the introduction of the health insurance system have been analyzed and determined.

I believe that the reliable correlation between the severity of epidemic outbreaks of trichinellosis through the Attack rate and the source of infection - domestic pig or wild boar needs statistical certainty.

Some scientific - applied contributions, which are summarized as follows, are worthy of attention:

1. The development and application of a laboratory ELISA IgG avidity test provides an opportunity to improve the diagnosis of trichinellosis and toxocariasis,

2. The clarifying of the profile of those affected by the study helminthiases provides valuable information for adequately targeting organizational measures for surveillance and control of these parasitoses.

3. Updated epidemiological data can be used as a scientific basis for the development of regulatory documents, management decisions and health network activities.

4. The results of the dissertation work can serve as a scientific basis for future programs in order to achieve a lasting reduction in the incidence of helminth infection in the country.

I believe that the results of this study should be brought to the attention of the responsible factors in health care, because they demonstrate the seriousness of the problem and the gaps in epidemiological surveillance and control in our country.

The abstract is written on 78 pages and includes a summary in English. Its content is in line with the thesis in the dissertation work.

Reflection of the doctoral student scientific results in literature

The publication activity of Assoc. Prof. I. Rainova is high and it should be noted that most of the publications are in reputable scientific publications with an impact factor. 25 publications related to the topic of the dissertation - 10 in international and Bulgarian scientific journals with IF were presented (common impact factor 13.369), 12 in non-impact factor editions and 3 in scientific congresses and conferences abroad. In 10 articles, Assoc. Prof. I. Rainova is the first author, and in 9 - the second author, which testifies to her leading participation in their planning, development and design. She participates with 5 chapters in the collective monographs and in 23 national and 9 international scientific forums with reports and posters. The 21 citations of 9 publications included in the documents show that part of the results of her dissertation have become available to the scientific community at home and abroad.

Critical notes and recommendations

The dissertation is well designed and no significant errors are identified. Through the remarks provided below, I would like to draw the applicant's attention only to some of the omissions that have no affect on my final assessment.

The summary would benefit if it not only reflect the results, but also includes their interpretation and conclusions. The English version of the summary could be better edited.

Due to the merging of the Results and the Discussion into one section, in some parts of the exposition there is an infusion of detailed literary data and own studies, which makes difficult to highlight the contributions of the author and give a more monographic character to the work.

Based on data from the study and current global practice experience, it would be useful to develop a modern diagnostic algorithm for trichinellosis.

Conclusion

The reviewed dissertation work deals with an important health and social problem concerning the most significant helminthozoonoses for Bulgaria - echinococcosis and trichinellosis, as well as the growing medical and public health significance of toxocariasis. The work has clearly formulated purpose and tasks, it has been developed competently, taking into account the latest achievements in the world literature in this field and applying modern research methods.

The scientific results are well analyzed, argued, documented and illustrated. The dissertation work is valuable not only in scientific-theoretical aspect, but it can find application in the practice for improving the epidemiological surveillance and control of helminth infection in Bulgaria. Publication activity in relation to the dissertation is high.

The dissertation fully meets the scientific criteria in the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Rules of the NCIPD for the award of the scientific degree "Doctor of Sciences". This gives me reason to vote convinced for the award of the scientific degree "Doctor of Sciences" in the scientific specialty "Parasitology and helminthology" to Assoc. Prof. Iskra Georgieva Rainova, MD, PhD.

13.01.2020

Prepared a review:

Prof. Rossitza Kurdova-Mintcheva, MD, PhD

