

REVIEW

By Assoc. Prof. Dimitar Ivanov Vutchev, MD, PhD

Section/ Parasitology and Tropical Medicine, Medical University - Plovdiv

Subject: Dissertation thesis for the award of the scientific degree "Doctor of Science"

Professional field: 7.1. Medicine

Scientific specialty: Parasitology and Helminthology

Author: Assoc. Prof. Iskra Georgieva Rainova, MD, PhD

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

1. General Overview of the procedure and the candidate for the scientific degree "Doctor of science"

- Dissertation /257/
- Abstract /78/
- List of scientific publications on the topic of the dissertation work/ 30 / - 5 chapters of monographs, 10 - in foreign and national journals with IF and 12 - without IF, 3 - in foreign books of abstracts
- A list of participating in scientific congresses / 9 abroad, 23 national /
- A declaration of originality and authenticity of the documents
- A list of citations /22 of publications / and total IF-13.369

2. Brief biographical information for the candidate. Assoc. Prof. Iskra Rainova was born in 1956 in Sofia, where she graduated in Russian Language High School (1974). She studied medicine in Medical Academy, Medical Faculty - Sofia, graduated in 1982 and then was appointed as a district pediatrician in Dupnitsa. Since 1985 she worked as a resident therapist at IV City Hospital, Sofia and in 1987 she joined NCIPD as a resident in DPTM and after a after competition she obtains a research fellow III-I degree (1991-1996) in the Laboratory of Experimental and Applied Parasitology. The specialty Medical Parasitology she acquired in 1992 and in 1996 – the specialty Clinical Immunology. In 2006 she defended her PhD thesis and in 2010 acquired the academic position Associate Professor of Parasitology and Helminthology. Since 2011, she was appointed as Head of DPTM, and since 2016 is appointed as a Deputy Director of NCIPD. Assoc. Prof. I. Rainova is a chief coordinator of the expert council on medical parasitology at the Ministry of Health. In 2002 she specialized in the diagnosis of parasitoses at the Institute of Parasitology in Bern, Switzerland. So far, under the direction of Dr. Rainova, 4 PhD students have defended their dissertations, two graduate students (biologists from the Sofia University), and currently she is the scientific

supervisor of one more PhD student. The main topics in her activities involve research, laboratory diagnostic, educational teaching, administrative, organizational and methodical functions to parasitological units in RHI. Assoc. Prof. I. Rainova is a member of the Bulgarian Medical Association (BMA), Bulgarian Society for Parasitology and the Bulgarian Association of Medical Parasitologists.

3. The relevance of the topic and the appropriateness of the objective and tasks set.

In the dissertation, the relevance of the prevalence data, epidemiological parameters, modern diagnostics and treatment of the main helminthoses - trichinellosis, toxocariasis and echinococcosis in the last two decades, is indisputable and objective. It is known that current data for echinococcosis distribution (the country is leading in morbidity in the EU) are unfavorable, outbreaks of trichinellosis repeated almost every year and studies reporting significant prevalence of toxocariasis in recent decades increased. All of this indicates that helminthozoonoses are an important medical and social problem for national healthcare and medical parasitology as a theoretical and applied discipline. The defined purpose of the dissertation involves a comprehensive study at a contemporary level, structured by problem-related three main sections of content for each parasitic disease, respectively prevalence and epidemiology, clinic and diagnosis, current therapeutic options. The objective is subordinated to specific scientific tasks that correspond to the sections cited. The topic identified and accepted for scientific development is based on long-term main epidemiological characteristics and their dynamics, clinical and diagnostic data, incl. development and implementation of new immunological and contemporary therapeutic approaches for practice. The final conclusions and contributions are logically followed, which are formulated briefly and clearly, focused theoretically and practically, and some of them are of a certain prognostic importance.

4. Problem knowledge /Level of knowledge of the state of the problem and the literary material /

As a longtime head of laboratory for experimental and applied parasitology at NCIPD and coordinator of the expert council of Parasitology at the Ministry of Health, Assoc. Prof. I. Rainova is deeply acquainted with the immunology of parasitoses on the one hand and on the other with the control and the state of the parasitic disease in Bulgaria. Thus, she has specifically focused on a comprehensive study of helminthoses of major medical and social importance. The doctoral student is mainly familiar with the modern specialized literature on Trichinellosis, Toxocariasis and Echinococcosis - in terms of prevalence and epidemiology, clinics and diagnostics, therapy and prophylaxis. The researched and used scientific sources testify to the clarity of the specific theoretical and applied problems defined for development and solving. The other authors cited in the literature review and the main text in that section of the dissertation are listed in the references. The Cyrillic alphabet contains 88 titles of scientific publications - separate publications and monographs by predominantly Bulgarian authors, and the remaining 339 - in Latin, mainly by foreign researchers. A total of 427 titles are listed, among them by authors with international authority in the relevant etiological area (helminth infection), as well as by prominent Bulgarian researchers.

5. Research methodology. The research methods are adequate to the main purpose and make possible the consistent and systematic solution of the formulated operational tasks in the dissertation. The author uses them excellent and gives them into different sections: 1) parasitology methods; 2) Serological methods - developed and implemented IHA (for trichinellosis and echinococcosis), ELISA IgG (for trichinellosis, toxocariasis and echinococcosis), confirmatory Western blot for specific IgG antibodies; 3) Epidemiological methods - determining basic analytical parameters in trichinellosis, toxocariasis and echinococcosis (incidence, prevalence, attack rate, mortality, lethality and standardized incidence). The dissertation work is shaped properly and thoroughly demonstrated. Quantitative data and other graphical indicators are documented and recorded in the 34 tables, 59 figures and one diagnostic algorithm. The essential components are processed by modern medical-statistical methods, using a specialized computer program.

6. Characterization and evaluation of the thesis. In summary - a study was conducted on the epidemiological, clinical, diagnostic and some therapeutic features of the most significant helminth infection in the country - trichinellosis, toxocariasis and echinococcosis - among the population for the period 2000-2017.

The first section - in the case of trichinellosis are tracked registered *Trichinella* outbreaks, infected persons consumed infected meat, and are calculated: incidence by year, age groups, sex and place of residence. Of note is the number of epidemic outbreaks investigated - a total of 96, out of 92 settlements, within 24 districts, respectively 2 068 persons were infected (55% of them were clinically pronounced, the others were asymptomatic). The significant mass of observation units provides statistical reliability of the results. The average incidence is 1.5‰, but it tends to decline over the years. Regarding the source of infection 45% of the outbreaks are due to wild boars, 40% of domestic pigs and with an unclear source are 15%, which raises concerns about the distribution of infected products from the commercial network, as it is indicated by the epidemiological studies. The highest number of outbreaks is traditionally recorded in winter (January and February), and the established long average incubation period of 28 days indicates a milder course of the disease (respectively, with lower intrusion). From the 1,146 persons with clinically pronounced trichinosis, 680 were admitted to hospitalization, mostly moderate and rarely severe. In this significant number of uncomplicated cases observed in hospital conditions, the clinical symptoms described are those of which the dominant characteristics are fever, swelling, myalgia and arthralgia, headache, diarrhea, allergic rashes, fatigue. From the serological examinations of 2,045 patients, 1 633 were positive and a possible negative result should not reject the admissible diagnosis in differential diagnostic plan. An important achievement for specifying the stage of infection (acute, chronic, latent) is the laboratory-developed ELISA IgG avidity test. The cost of hospitalization of patients with trichinellosis (by clinical pathway) was calculated - 153 121 BGN, in outpatient care it was 180 820 BGN.

The second section includes studies of toxocariasis in humans (*Larva migrans visceralis*). While numerous scientific studies have been carried out for trichinellosis and echinococcosis by diverse specialists, the research of Assoc. Prof. Rainova on toxocariasis in

humans is pioneering and impressive in its depth and versatility. The data she receives is unique to the country, quite often of general scientific importance. According to the clinical status, the following forms are conditionally distinguished: visceral, ocular, neurological, dermatological and asymptomatic, with leading eosinophilia in the blood (8-77% of the studied). The most commonly studied serologically are those with allergic symptoms and accompanying eosinophilia. Serologically tested were 2 087 persons, of which 13.4% had a positive result. Alarming is the large part of positive children aged 1-4 years (13.7%) as well as adults of working age - 45-49 years (10%). With clinical data on toxocariasis, a total of 1,228 individuals were tested. For latent invasion, the author conducted a sero-epidemiological screening of 550 children and adults, including a surprising diagnosis of 6.7% of seroprevalence. An important result of immunological studies is the determination of the role of eosinophilic cationic protein as a possible marker indicating the stage of infection. Serological tests ELISA and Western blot were also analyzed, and an ELISA IgG avidity test was elaborated to determine acute or chronic toxocariasis. At the end of the section is elaborated, a diagnostic algorithm, designed mainly for the medical practice in suspected and confirmed cases of toxocariasis.

Section 3 includes extensive studies of echinococcosis in our country. The author found the morbidity for the study period at the beginning of the 21st century to be 5.4%, and the incidence was 6.04%. An impressive number were registered - 8157 reported cases of echinococcosis, and a significant number of recurrent ones - 882 (11%). Individuals in active working age are affected mainly (20-39 years) - 30.6%, but the high proportion of children and adolescents (1-19 years old) - 20.2% is particularly worrying. The latter indicator explicitly showed the existence of active parasite sources and the maintenance of transmission of the infection. More villagers were affected (10.7‰), but no citizens were spared (4.1‰). From echinococcosis and its risk complications (rupture with allergic shock) died 175 patients. Lethality was 2.14% and it is obtained in indigenous parasitoses only for echinococcosis.

In 70% of patients, the echinococcal cyst is located in the liver, followed by pulmonary localization (18.8%, more common in children), and multiorgan localization (6.5%), in the CNS, lien, kidneys, bones, muscles, subcutaneous, eyes, heart, etc. From clinical laboratory tests, eosinophilia is observed only up to 20% of patients, leukocytosis - rarely, if there are complications (abscess). Serodiagnosis is used almost universally. A positive result in ELISA and IHA was reported in most patients, but 542 were negative, regardless of the parasitic finding. The application of the confirmatory Western blot test to evaluate its effectiveness was examined. In terms of therapy, surgical treatment was applied in 7326 patients, 103 were treated with PAHR, and postoperative chemoprevention of recurrence with albendazole was administered in 2132 cases.

After the three main sections of the complex studies, Assoc. Prof. Rainova makes an objective self-assessment of the contributions, which I accept unreservedly and answers the question of what should be done. I quote freely: "... studies of helminth infection have updated and summarized the data over the past two decades regarding major

epidemiological, clinical and laboratory diagnostic parameters.” Despite the downward trend in the incidence of trichinellosis and echinococcosis, the country remains one of the leaders in the European region. The results of the overall study show that the control measures against helminthozoonoses should continue and the link between responsible institutions strengthened.

7. Contributions and importance of the dissertation work to science and practice.

The contributions (11 issues) are grouped substantially into three sections, each of them of specific theoretical and practical importance, summarized respectively. such as:

I. Original contributions (4). In the dissertation work was performed detailed analyzes of the studied helminthozoonoses in the last two decades, with epidemiological characteristics in the dynamics of trichinellosis, toxocariasis and echinococcosis. These studies are a basis for planning targeted surveillance and control. A correlation was found in the extent of trichinellosis epidemic outbreaks with the Attack rate and source of infection (wild boar and domestic swine). To assist in the diagnosis of trichinellosis and toxocariasis, an ELISA IgG avidity test has been elaborated to determine the chronicity of the immune response - important for refining the patient's clinical condition, and therapeutic behavior respectively. The doctoral student has made a calculation that indicates the significant medical and financial costs for trichinellosis.

II. Scientific and applied contributions (5). With the studied dynamics of morbidity and mortality in helminthozoonoses, downward trends have been outlined - we accept them as a positive prognostic indicator, but definitely insufficient. The etiologic diagnosis of trichinellosis and toxocariasis has been improved with the introduction of the ELISA IgG avidity test developed by the doctoral student. Major population groups at higher risk of echinococcosis infection have been identified, to which epidemiological surveillance and control should be strategically planned and targeted. The results of the epidemic analysis of helminthozoonoses are determined by the author, who I strongly support, as a sound basis for continued scientific research and activation of disease reduction and control measures at regional and national level.

III. Confirmatory contributions (2). We support the conclusion that the repeatability of trichinellosis outbreaks indicates a low level of health education and requires targeted health promotion, especially among populations at risk. The list of symptoms and syndromes, the results of hardware and imaging diagnostics, the chapel in the parasitoses studied is confirmed and supplemented by the clinical observations. Particularly necessary for general clinical practice is the compiled Clinical and Diagnostic Algorithm for toxocariasis in humans. The dissertation ends with 22 generalized conclusions concerning the scientific and practical achievements of the author, which are reflected in the text of the dissertation and in synthetic form are given in the contributions section.

8. Publications. In section publications and reports in scientific forums were announced five chapters in scientific monographs, as well as 10 publications in Bulgarian and foreign journals with IF (total IF 13 369). There were 12 publications in non-IF journals, 9 articles in collections of scientific forums abroad, 23 submissions with reports in national

scientific forums. Nine different publications were cited, with the collective participation of the author in a total of 22 scientific publications - foreign and Bulgarian.

9. Personal participation of doctoral student. The studies, included in the dissertation, with formulated results and contributions, adequately meet the set purpose and tasks. They have been carried out, processed and achieved with the personal participation of the doctoral student - as a leader and performer. Assoc. Prof. I. Rainova participated in the development of 4 scientific projects (one international), led by Prof. P. Petrov and Prof. R. Kurdova, funded by the Ministry of Education and Science and related to the development and creation of new immunodiagnostic, molecular-biological methods and sero-epidemiological studies in parasitoses of medico-social importance, incl. trichinellosis, toxocariasis and echinococcosis.

10. The abstract is well prepared, and constructed according to the dissertation with relevant sections, contains representative data from the research studies, and is illustrated professionally and sufficiently. The abstract provides synthetically the content of the work, the conclusions, contributions and additional information.

11. Critical remarks and recommendations. The dissertation is prepared thoroughly and I have no comments. Doctoral student has accepted and complied with the recommendations of the internal defense.

12. Personal impressions. In the dissertation, the author has elaborated a huge amount of material - reliable, originating from all over the country and processed statistically, also from personal laboratory research; routine and modern methodologies are applied, and the results obtained are complete in theoretical and applied terms. It is important for the anti-epidemic and diagnostic-therapeutic practice. The reviewed work gives the impression of a serious and thorough approach to the scientific search and points to great creative potential and successful work at DPTM - NCIPD, the only highly specialized complex structure for medical parasitology in the country.

13. Recommendations for future use of dissertation contributions and results. The formulated conclusions from the developed work are objective, sufficiently substantiated and necessary grounds for the preparation of a new National Program for the control of echinococcosis - with a tendency to its elimination, as well as for the improvement of diagnostics, respectively. treatment and restriction of toxocariasis and trichinellosis. The compiled algorithm for the diagnosis of toxocariasis should also be published, with the aim of using it from the medical practical network.

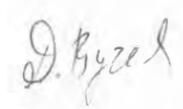
CONCLUSION. The dissertation contains scientific, scientifically applied and practical results, which represent a contemporary contribution to the national parasitological science and practice and meet the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the Rules of the NCIPD. The data and the results of the study presented in the dissertation correspond to the specific provisions of the law. The dissertation shows that the doctoral student Assoc. Prof. Iskra G. Rainova, MD, PhD possesses in-depth theoretical and practical knowledge,

professional qualification, as well as the qualities of a performer and leader to a research team, with significant realized scientific and applied achievements.

Taking into account all of the above, I am convinced of my positive appreciation of the research and studies reflected in the peer-reviewed dissertation, results achieved and contributions, with a proposal to the Honorable Members of the Scientific Jury, appointed by the Director of the NCIPD, Assoc. Prof. Iskra Georgieva Rainova, MD, PhD to be awarded the science degree "Doctor of Science" .

Date: 18/01/2020

Reviewer:

A handwritten signature in black ink, appearing to read "D. Vutchev", is positioned above the printed name of the reviewer.

Assoc. Prof. Dr. D. Vutchev, MD