To the honorable members of the Scientific Jury
Designated by order No. 510/16.12.2019
Of the Director of the National Center
for Infectious and Parasitic Diseases
Prof. Todor Kantardjiev, MD, DSc

Statement of Opinion

By Assoc. prof. Lyubka Yordanova Doumanova, PhD
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On the PhD thesis submitted by Silvia Emilova Voleva
for defense of the educational and scientific degree "Doctor" in the scientific specialty "Virology" in the professional field 4.3. Biological Sciences

Title of the thesis: "Serological and molecular genetic study of the prevalence of parvovirus B19V in pregnant women and women of childbearing age in Bulgaria"

The present PhD thesis refers a 5 year study which monitors through serologic and molecular genetic methods the circulation and spread, as well as the intensity and the clinical aspect of parvovirus B19 (B19V) infection among pregnant women, risk and pathological pregnancy included, women with fertility problems, and newborns, as well.

It is a well-known fact that, worldwide, viral infections in pregnant women are a major cause of complications and mortality, with B19V being one of the important but widely neglected viral agents. Therefore, the search for opportunities to introduce and promote screening programs and to develop modern approaches for the rapid and reliable diagnosis of infections with this important pathogen is a pressing issue. The topic is also a pragmatic one with a practical target for specialists in obstetrics, because one of the main author's contributions is the development of a diagnostic algorithm for pregnancy monitoring when B19V infection is present.

The so formulated aim is informative, clearly and precisely combining the directions of the experimental work. To achieve the goal, five specific and relevant research tasks are assigned: (i) detecting B19V infection in the sera of different target groups of women; (ii) detecting B19V nucleic acid in clinical specimens and determining the etiological role of B19V in the development of certain clinical syndromes during pregnancy; (iii) performing genetic analysis of B19V sequences; (iv) comparing the results from the serologic and

molecular genetic studies and (v) ultimately developing an algorithm to detect specific B19V markers in women with pathological pregnancy. The adequacy of the defined main goal and the fulfillment of the research tasks are a prerequisite for the good scientific results and contributions obtained.

The literature review serves as a stable basis for formulating the goal and objectives. The review is tight, well-shaped and sharing enough factology. This part of the thesis demonstrates that the PhD student is well orientated in the literature on the general characteristics, structural and genomic organization and replication of B19V, the epidemiology, clinical manifestation and pathogenesis of parvovirus infection. This awareness also helps in the discussion part to correctly interpret the data received. Reference list comprises 358 items, and one of them is in Cyrillic. References are completely up-to-date – 35% of them are published in the recent ten years and among these 28% are published in the last 5 years. Out of all 358 references, 10% are published the last 5 years. This confirms the author's good and contemporary knowledge on the problem.

The research is properly planned from a methodological point of view. This is a prerequisite for the correct conduct and execution of the experiments and the reliability and reproducibility of the results obtained. The methods used are described in an informative way, with the introduction of the necessary controls and an indication of the kit used with the appropriate protocols. This indicates that the PhD student has successfully mastered and applied basic serology methods (indirect immuno-enzyme method), molecular biology methods (standard and nested -PCR, sequential analysis of positively amplified NS1/VP1 products, sub-genotyping and phylogenetic analysis), and application of various software programs for a large set of statistical research methods, which are tailored to the specific experiments and are adequate in relation to the tasks. In prospectively studied pregnant women, women in childbirth, in women who suffered spontaneous abortion and such who underwent *in vitro* procedures, informed consent form has been filled-in in accordance with present legislation.

An essential part of the dissertation is the Results section which is structured in alliance with the research tasks set. The results are illustrated by 10 figures and 14 tables, accompanied logically with analysis and short discussion. As a separate part is a chapter entitled "General discussion". In this chapter particularly, the author reveals an acquired ability to make thoughtful, thorough, critical comments, which also compare the results with literary sources. However, this way of exposing the discussion hides a risk of iterance.

Silvia, although a PhD student, is already an established specialist, knowing in depth

the problem,who formulates and provides to the scientific audience an own opinion. This is expressed in 8 conclusions and 3 original contributions. I accept undoubtfully the conclusions and contributions thus formulated. They are relative to the goal of the study, and correspond to both the goal and the research tasks, as well as to the results presented and discussed.

The obtained results are published in 10 scientific papers with a total IF of 3.651 and in 8 of them Silvia Voleva is the first author. According to the SJR metrics, seven of the journals possess Q4 quartiles and one – Q3. There is an abstract published in a journal with IF, which I only accept as an abstract, not a full-text paper. Presentations on various international and national scientific forums with posters and papers related to the topic of the PhD thesis are 12 in number which is very impressive. There are two research projects that partially finance the topic of the dissertation. No citations are provided.

In conclusion: Following my analysis of the PhD thesis of Silvia Voleva, I believe that in its content, methodological level, design, relevance, practicality and relevance, it represents a thorough and substantiated scientific study with credible results, clear conclusions and real contributions outlined in the field of virology. It meets the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria and the Regulations for its implementation, as well as the Rules of the NCSPD for the acquisition of the educational and scientific degree "Doctor". Silvia Voleva fulfills also the minimum national requirements, as well as the additional quantitative criteria, as adopted by the National Center for Infectious and parasitic Diseases.

I definitely provide my positive assessment and recommendation to the distinguished Scientific Jury to award the educational and scientific degree "Doctor" of Silvia Emilova Voleva.

February 9th, 2020

Sofia

Opinion prepared by:

Assoc. prof. Lyubka Doumanova, PhD