To the members of the scientific jury
of the Director of NCIPD

POSITION

Of Prof. Dr. Iva Stefanova Hristova, MD, Ph.D.

Concerning Dissertation thesis for the award of the educational and scientific degree
"Doctor" in the scientific specialty "Virology"
in the professional field 4.3. Biological Sciences

Thesis topic:
STUDIES ON DISTRIBUTION IN BULGARIA AND GENETIC
CHARACTERISTICS OF HUMAN METAPNEUMO VIRUSES, PARAINFLUENZA
VIRUSES AND BACAVIURIES

Author of the thesis: Ivelina Trifonova Trifonova, Department of Virology, NCIPD

Ivelina Trifonova's dissertation has several merits. First, the subject of the study is very relevant, these are respiratory viral infections that are ubiquitous, severe in infants and appear annually as reinfections. Second, studies on three viral infections have been carried out, which is a huge work. Third, modern molecular virological techniques for phylogenetic identification, typing and sequencing have been applied.

Excellently conducted, these studies show the current etiologic structure, geographical distribution, epidemiology and clinical forms of acute respiratory infections caused by human metapneumovirus, parainfluenza and bocaviruses. The phylogenetic analysis revealed the viral genotypes circulating in our country.

Based on an impressively large number of literary sources (n=358), Ivelina Trifonova makes a thorough and competent literary review on 50 pages. The review is written very well, comprehensively and concisely. Human metapneumovirus,
parainfluenza and bocaviruses - their classification, structure, epidemiology, pathogenesis, clinic, treatment, virological diagnostics are complexly described.

The aim of the dissertation is substantiated and 7 tasks are formulated clearly and precisely. The Materials and Methods section, with a total volume of 26 pages, describes the patients and clinical samples examined. The methods of viral RNA extraction, primer pairs, probes and conditions for conducting real-time polymerase chain reactions are described, as well as methods for phylogenetic analysis based on nucleotide and amino acid analysis.

The results of her own research are presented on 62 pages and discussed on 14 pages. They are illustrated with 39 tables and 26 figures. Description of the results is accurate, clear and demonstrates the excellent preparedness of the doctoral student.

Presentation of the results follows the logical sequence of the tasks assigned. The etiological structure of viral acute respiratory diseases in Bulgaria, 2016-2019 was studied. It has been found that human metapneumovirus, parainfluenza viruses and bocaviruses cause 6.8%, 6.3% and 8% of these infections respectively. Among parainfluenza viruses, type 3 is proven with the highest frequency, with less prevalence of type 2 and type 4.

Seasonal activity of the three viruses was studied. It has been found that human metapneumovirus is most commonly detected in winter and spring, bocaviruses are detected throughout the year, most often in the fall and early winter, and type 3 parainfluenza viruses circulate throughout the year with the exception of the summer months.

The age structure of patients affected by the three types of viruses was investigated. Human metapneumovirus, parainfluenza viruses and bocaviruses have been found to be most commonly detected in children aged 0-4 years. Human metapneumovirus causes disease in patients of all age groups. Parainfluenza viruses and bocaviruses have not been demonstrated in patients in the middle-aged group.

Participation of the studied viruses in co-infections was investigated: bocaviruses were found with the highest incidence in mixed infections and metapneumovirus with the lowest.

Analysis of clinical syndromes caused by the viruses studied indicates that human metapneumovirus and bocaviruses more commonly cause bronchiolitis and
pneumonia in children, whereas parainfluenza viruses cause predominantly laryngotracheitis and bronchiolitis.

A serious merit of the thesis is phylogenetic analyses of gene F of human metapneumoviruses and of gene VP1 / VP2 of human bocaviruses. In addition, amino acid analyses show degree of variability of the genes examined.

An excellent discussion of the findings was made in the light of previous studies by other Bulgarian, European and international authors.

Substantial scientific contributions have been made. The main among them are the first studies in Bulgaria of recently discovered human bocaviruses, studies on parainfluenza viruses by modern genetic methods, and studies on human metapneumoviruses of a large number of clinical samples.

The results of the dissertation are published in 5 journal articles, 2 of which are in prestigious foreign publications (J Med Virol, Braz J Microbiol) with a high impact factor (total impact factor 4,957). The data were also presented at 20 scientific conferences, 7 of which were abroad. Ivelina Trifonova is the first author of 3 of the 5 publications and 9 of the 20 presentations in the scientific forums related to the dissertation, which is the evidence of her major involvement in their implementation.

In conclusion, Ivelina Trifonova's dissertation focuses on little-studied viral pathogens of acute respiratory infections, mainly in children up to 5 years of age. Planned and meticulously executed, this dissertation has made significant contributions, as evidenced by the publications. The PhD student has mastered a wide range of modern methods, thoroughly analyzes the results and reaches summaries and conclusions.

I believe that the reviewed dissertation fully meets the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria, the Rules for its implementation and the Rules of the NCIPD for its implementation. Giving my positive opinion, I propose all the members of the scientific jury to award Ivelina Trifonova Trifonova the educational and scientific degree "Doctor" in the specialty Virology.

Sofia
02/01/2020

Signature: [Signature]
(Prof. Dr. I. Christova, MD)