

БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ  
И-Т ПО ЕКСПЕРИМЕНТАЛНА МОРФОЛОГИЯ,  
ПАТОЛОГИЯ И АНТРОПОЛОГИЯ С МУЗЕЙ  
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СОФИЯ

To the Chair of the Scientific Jury  
Determined by order  
№ RD-09-07/07.02.2022  
of the Director of IEMPAM-BAS  
Sofia

## REVIEW

Under a competition for the academic position "Associate Professor" in the professional field 6.4. Veterinary medicine, scientific specialty 04.03.06 "Animal Pathology", for the needs of the section "Pathology" at the Institute of Experimental Morphology, Pathology and Anthropology with a Museum (IEMPAM), announced in SG no. 107 / 16.12.2021 with the only candidate Assistant Professor Dr. Katerina Stanimirova Dimitrova.

**Prepared by:** Professor Hristo Miladinov Naidenski DSci, Corr.-Member of BAS from the Stephan Angeloff Institute of Microbiology at BAS. Competence in infectious microbiology, molecular biology, immunology, epizootology, experimental animal models.

I declare that there is no conflict of interest between me and the candidate Assis. Prof. Dr. Katerina Stanimirova Dimitrova within the meaning of Paragraph 1, items 2a, 3, 4 and 5 of the Additional Provisions of the Law on the Development of the Academic Staff in the Republic of Bulgaria. The documents submitted to me under the competition for preparation of a review have been correctly prepared and comply with the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its application in IEMPAM-BAS.

### 1. Brief biographical data and characteristics of the applicant's scientific interests.

The candidate for the academic position "Associate Professor" Dr. Katerina Stanimirova Dimitrova, PhD, was born in 1979 and graduated in 2005 from the University of Forestry in Sofia, Faculty of Veterinary Medicine with a Master's degree and a Veterinary Qualification. In 2015 she successfully defended her PhD dissertation on "Pathomorphological and immunological studies in chickens experimentally treated with fumonisin B1" and obtained the educational and scientific degree "Doctor". In the following years, Dr. Dimitrova upgraded her research experience in the field of cell biology, morphology, clinical and experimental pathology, and infectology. The specializations in foreign research units in Germany (Institute of Medical Immunology, Berlin) and Italy (University of Pavia) also have a very positive effect on her professional development.

All these brief biographical data testify to the formation of lasting scientific interests in current biomedical fields and clearly outline the profile of Dr. Dimitrova as a promising researcher in the field of cytopathology, experimental oncology and implantology.

## **2. General description of the presented scientific production.**

In this competition the candidate presents a total of 53 scientific publications in specialized scientific journals, which are distributed as follows: journal articles in journals indexed in WoS, Scopus, ERIH+, a total of 31, and articles in non-indexed journals in WoS, Scopus, ERIH+, thematic collections, incl. proceedings of international and national scientific forums - 22.

Particularly impressive are the publications in the journals like European Polymer Journal, Biomedicines, Viruses, Materials Science and Engineering C, Acta Biomaterialia and others. The participation of Dr. Dimitrova in the presented scientific articles is emphasized - in 18 she is a leading author, in 9 she is a second author, in 16 a third, etc. To the scientific assets should be added the serious participation of the candidate with a total of 61 presentations at scientific forums, 55 of which in national scientific forums, including national ones with international participation and 6 in international scientific events.

## **3. Evaluation of research and applied scientific activities.**

The overall research activity of Dr. Katerina Dimitrova is focused mainly on the search and development of new approaches and strategies for diagnosis and treatment of a number of infectious and non-infectious diseases, as well as to clarify the factors and mechanisms responsible for initiating and developing of the pathological processes. Very professionally and precisely she uses well-established experimental cell lines (tumor and conventional), as well as animal models to assess biological activity (antitumor, bactericidal, antiparasitic, immunomodulatory, etc.) and reveal the mechanism of action of many studied natural and synthetic products. The obtained original scientific results are the basis of many contributions of fundamental and scientific-applied significance.

In one of the research areas the biological properties of a number of plant extracts and hemolymph products of invertebrates in *in vitro* and *in vivo* conditions have been studied. The extracts of the plant *Cotinus coggygia* have been shown to demonstrate the most pronounced antiproliferative effect and by this way should be a potential therapeutic agent in cases of cancer disease. The lowest toxicity accompanied by tissue-protective activity was found in the ethyl acetate extract of *C. coggygia* leaves (publications №№ 15, 20, 23, 28). The  $\alpha$ -HaH and  $\beta$ c-H1H subunits of hemocyanins isolated from the snails *Helix aspersa* and *Helix lucorum* are also promising for the development of new therapeutic agents for the treatment of bladder cancer (Publication № 18).

Precise cytomorphological analysis of cells from the line HT-29 treated hemocyanin found that the observed antitumor effects of *Helix aspersa* and  $\alpha$ -HaH mucus are associated with the induction of apoptosis in tumor cells, which revealed the potential for developing new therapeutic agents for the treatment of colorectal cancer (publication № 14). The carotenoid astaxanthin extracted from *Coelastrella* BGV microalgae also has antiproliferative activity demonstrated *in vitro* by apoptotic cell death in HeLa cells (Publication № 27).

In this direction, the biological properties of a number of synthetic products have been studied *in vitro* and *in vivo*. A series of studies have examined the effect of the use of inorganic or organic compounds of zinc and iron in compound feed for industrial broiler breeding, as well as concentrations at which no side effects are observed (publications №№ 2, II-6, II-7). The highest weight at the end of the period was observed in broiler chickens treated with zinc methionate (Zn-Met) 60 ppm - 2.013 kg compared to birds treated with iron methionate, iron sulfate, and zinc sulfate heptahydrate.

Studies on erufosine (synthetic alkylphosphocholine analogue called erucylphospho-N,N,N-trimethylpropylammonium, ErPC3) have demonstrated a significant antitumor effect in a model of hamsters with induced myeloid tumor of Graffi (publication № 19) and revealed by inhibition of tumor growth, suppression of metastatic activity and prolongation of the average survival time of experimental hamsters. Of great medical importance and relevance are studies on diabetic mouse model of newly synthesized multifunctional hydrogels based on thiolated hyaluronic acid (HA-SH) and bioactive silver-lignin nanoparticles (Ag@Lig NPs) for treatment of chronic wounds. By inhibiting the enzymes myeloperoxidase and matrix metalloproteinases responsible for wound chronicity, as well as after the release of antimicrobial silver in response to increased infection-related hyaluronidase activity, complete tissue regeneration and skin recovery is achieved. In this direction are the studies on poly (carboxybetaine methacrylate) hydrogels, which are characterized by high absorption capacity in swelling in saline solutions and reveal great potential as dressings for highly secretory chronic wounds. Hydrogels do not show cytotoxicity and prevent the formation of biofilm from *S. aureus* (publication № 16).

Antimicrobial and antibiofilm-forming activity has also been demonstrated in zinc oxide nanoparticles (ZnO NPs) applied to silicone urinary catheters in a 7-day rabbit model. The results of clinical, haematological and histological examinations of the urinary system indicate that this strategy could be successfully applied in clinical practice for the prevention and control of catheter-related urinary tract infections (publication № 24). Data on the biocompatibility of polymer hydrogels ( $\beta$ -tricalcium phosphate implants ligated with Zn/Mg, polyzwitterionic hydrogel systems, etc.) have potential for application in surgery and dentistry (publications №№ 12, II-9, II-10).

The second important focus of Dr. Dimitrova's research is the pathological and clinical aspects of infectious, non-infectious and parasitic diseases in humans and animals. An extensive study of 100 patients with autoimmune thyroiditis after thyroidectomy examined the role of human herpesvirus-6 (HHV-6) in the development of this autoimmune disease. Markers for active infection were detected by RT-PCR with three different HHV-6 gene targets (U79 / 80, U51 and U12), and by immunofluorescence microscopy for expression of HHV-6 antigen and chemokine RANTES (Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted) in thyroid tissue, HHV-6 has been shown to persist in thyrocytes and interact with this chemokine (publications №№ 6, II-13, II-14, 17).

Field studies on BLV (Bovine leukemia virus) and HTLV1 (Human T-lymphotrophic virus type 1), belonging to the genus *Deltaretrovirus*, family *Retroviridae*, have established the ability to cross-react in humans and cattle. In addition, BLV has also been shown to infect rabbits and rats treated with whole blood or serum derived from leukemic cows, making these two species of laboratory animals, and especially rats, a suitable model for leukemogenesis due to BLV / HTLV infections (publications №№ 1, II-1, II-2).

Ultrastructural and immunohistochemical studies on calves with coronavirus pneumoenteritis syndrome reveal some features of the pathogenesis of the disease, as well as monoinfection with coronavirus in 20% of animals, and in the remaining 80% of cases coinfection with other pathogens - bovine rotavirus, *Escherichia coli*, *Clostridium perfringens* and *Cryptosporidium parvum* (publication № 9).

Dr. Dimitrova's research on the causes of a number of parasites is also in-depth and original. Using modern immunohistochemical and molecular biological methods (labeling with gold nanoparticles conjugated to lectins, RT-PCR, etc.) it was monitored the dynamics and distribution of *Trichinella spiralis*, as well as the expression of enzymes of the sialyltransferase family during the muscle phase of the trichinelosis (publications №№ 8, 10, II-12). With important contributions to the pathogenesis, clinical course and therapy are the studies on various parasitoses caused by the genera *Metastrongylus*, *Passalurus*, *Nematodirus*, *Eimeria* and *Ascarops strongylina*, *Macracanthorhynchus hirudinaceus*, *E. magna*, *E. media* and *E. perforans* in domestic animals and game (publications №№ 11, II-18). Mycotoxicological studies on the effects of fumonisin B1 (FB1) and deoxynivalenol (DON) on the immune system of chickens are also original in their nature. Complemented by histological and ultrastructural studies of lymphoid organs, it has been shown that FB1 and DON applied in natural concentrations, and especially their combination, affect the structure and function of lymphocytes, macrophages and immune system organs, as well as the health and immune status of poultry in general, with a possible adverse economic outcome (publications №№ 3, II-3).

Excellent knowledge of the basics and the capabilities of a number of molecular biological, biochemical, immunological, cellular biological, virological, histopathological, morphological and others methods have made scientific contributions of fundamental importance to industrial livestock and game breeding, and the protection of human and animal health e.g. food safety (publications №№ 21, 22, 26, 29, 30, II-20, II-21).

#### **4. Reflection of the candidate's scientific activity in Bulgarian and foreign literature.**

The purposeful and active research activity of the candidate on current issues in the field of biomedicine has resonated among the scientific community in Bulgaria and abroad. The candidate's articles have been cited 44 times, which testifies to the research interest shown in

the published results. It should be noted that the articles are cited mainly by foreign authors (41 citations) and in publications of prestigious international journals with a high impact factor.

#### **5. Participation in the implementation of research projects and contractual tasks.**

Dr. Dimitrova's active research activity also determines her fruitful work on a total of 23 research projects, in 11 of which she is the head of working groups. An important role for her professional development is her participation in two projects under the Operational Programs of the EU Structural Funds "Support for the development of PhD students, postdocs, postgraduates and young scientists" and "Building and developing of young highly qualified researchers and teachers for innovative interdisciplinary research benefits for biomedicine". Her participation in projects with Bulgarian (3 in total) and foreign universities is significant - 5 projects. The participation of Dr. Dimitrova in 3 projects financed by the Research Fund at the Ministry of Education and Science of the Republic of Bulgaria and 4 projects financed by the business is active. She has also successfully performed research tasks in one project under the 7th Framework Program of the EU, one under COST ACTION No. 15111 and two under bilateral international cooperation within the framework of inter-academic treaties and agreements of the Bulgarian Academy of Sciences.

#### **CONCLUSION**

The overall sixteen years of research activity of Dr. Dimitrova is focused, ascending and related to the acquisition and application of a wide range of modern methods for studying experimental or clinical cases of infectious, non-infectious, oncological and toxicological diseases in humans and animals. The overall research activity presented by the candidate is not only significant and up-to-date, but also a reliable platform for increasing the diagnostic capacity and expert potential of IEMPAM. The experience gained, active and fruitful work in the above areas (which are still in the focus of scientific research and professional interests) will undoubtedly contribute to more effective therapy and prevention of the above diseases.

Dr. Dimitrova's proven research skills are a reliable platform and guarantee for successful research in the field of biomedicine and in the future. Fully meeting the minimum requirements of the Law for development of the academic staff in R Bulgaria, as well as the required criteria of the Institute of Experimental Morphology, Pathology and Anthropology with

a Museum at BAS, and given all the above arguments, I propose to the esteemed Scientific Jury to award Assistant Professor Katerina Stanimirova Dimitrova, PhD, the academic position "Associate Professor" in the professional field 6.4. Veterinary medicine, scientific specialty 04.03.06 "Animal Pathology", for the needs of the section "Pathology" at the Institute of Experimental Morphology, Pathology and Anthropology with a Museum.

April 15, 2022

Sofia



Prof. Hristo Naidenski, Dsc., Corr.-Member of BAS