

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

Review

from **Prof. Dr. Ivaylo Stefanov Stefanov**, Doctor of Veterinary Medicine, Faculty of Medicine, Thrakia University, Armeyska Str. 11, 6000 Stara Zagora, member of the Scientific Jury of the competition for the academic position "Associate Professor" under Order 09-07 / 07.02.2022 of the Director of the Institute of Experimental Morphology, Pathology and Anthropology with Museum (IEMPAM) at BAS, Sofia.

Subject: Conducting a competition for the academic position "Associate Professor" in the scientific specialty "Animal Pathology" with code 04.03.06, in the field of higher education 6. Agricultural Sciences and Veterinary Medicine, professional area 6.4. Veterinary Medicine, "announced in the State Gazette, issue 107 of 16. 12. 2021. for the needs of the Department of Pathology at the IEMPAM - BAS.

Materials for participation in the competition have been submitted by Chief Assistant Dr. Katerina Stanimirova Dimitrova only, Department of Pathology, IEMPAM - BAS, Sofia.

The set of materials presented by the applicant is in accordance with national regulations for academic positions and comply with the specific requirements of the regulations of the IEMPAM – BAS and include all required documents.

I. Career profile of the applicant

Chief Assistant Dr. Katerina Stanimirova Dimitrova graduated in Veterinary Medicine, in 2005, at the Faculty of Veterinary Medicine, University of Forestry- Sofia with a qualification "Master of Veterinary Medicine". In 2005, she joined IEMPAM - BAS as a technical assistant, after which she was selected as a Research Associate of 3rd degree. During the period 2010-2022, the candidate held consecutive positions of assistant and chief assistant.

Chief Assistant Dr. Katerina Dimitrova received the Educational and Scientific degree "Doctor" in Pathology of animals after defending a dissertation on "*Pathomorphological and immunological studies in chickens experimentally treated with fumonisin B1*" in 2015.

The candidate's career growth includes active research work in several main morphological areas: research in the field of pathological and clinical aspects of diseases in humans and animals of infectious and non-infectious nature, study of biological activity and safety of newly synthesized, nanostructured materials or natural products by combining both in vitro and in vivo approach for their medical application as well as studies in the field of cytopathology, experimental oncology and implantology.

An important part of Dr. Dimitrova's career development is her participation in 23 research projects funded by the national and foreign Research Funds, companies and universities. It is noteworthy that Dr. Dimitrova has mastered various classical and modern methods of research that guarantee accurate results, allow detailed analysis and original contributions, which are not only fundamental but also scientifically applied.

These methods include cytological methods (BALB /c 3T3 NRU-test for cytotoxicity, MTT Dye Reduction assay-test for determination of the antiproliferative effect, Trypan blue test, flow cytometric studies; fluorescent methods: cytomorphological studies of cells after normal double fluorochromium with acridine orange and propidium iodide, staining with DAPI, Annexin V-FITC and propidium iodide (PI). Clinical in vivo studies of different types of laboratory animals have been performed, a standard method have been used to study the blood picture and blood biochemistry as part of the diagnosis of diseases. Classical and modern methods for light and electron microscopic examination of tissues and organs, immunocyto- and histochemistry, immunofluorescence, etc. have also been performed. It is important to note that histochemical studies have been performed on different histological or cytological material: cadaveric, from animals and humans, postoperative material, fingerprints, smears, tissue and cell cultures.

The use of statistical methods for data processing allows precise interpretation of the scientific results, detailed comparative analysis of the available literature and important conclusions as well.

The obtained original scientific results have been published in peer-reviewed international journals with a significant Impact Factor.

II. General description of the materials submitted in the competition

Chief Assistant Dr. Katerina Dimitrova presents for the review in the competition a total number of scientific papers - 53 articles published in full text in Bulgarian and in English, of which, 31 articles were published in refereed journals, 18 were published in journals with IF (some with very high IF = 7.328 and 8.947) and 7 - in journals with SJR. The personal contribution of the applicant is certified by his participation in 18 publications, where he is a leading author but in 8 publications he is a second author.

A list of 44 citations in Bulgarian and foreign specialized scientific journals was attached.

Dr. Katerina Dimitrova has participated in 23 national and international funded research projects, one of which she is a Leader.

She participated in 61 national and international scientific forums, 6 of which abroad and 55 in Bulgaria. Most of the scientific papers were presented orally, and in 25 of the presentations she is the first author.

Dr. Katerina Dimitrova applied a list of 12 specialized courses for mastering the application of various research methods, which she confidently uses in her studies.

The two specializations, respectively in Italy, Pavia, "Training school on-omics and bioinformatics in ME / CFS research", Department of Earth and Environmental Sciences, University of Pavia, COST action CA15111. 14. and in Germany, Berlin, "Summer school for translational research in ME / CFS", Institute Medical Immunology, Charite Campus Virchow Klinikum, COST action CA15111 enable the applicant to improve her knowledge and skills in conducting variety of studies.

Evaluation of the candidate's scientific works.

Dr. Katerina Dimitrova attached a very well-prepared detailed report on the contributions of scientific works, which accurately reflects her achievements in various scientific fields of fundamental and scientific-applied importance. The main scientific contributions of the candidate are related to:

- *Study of the biological activity of natural and synthetic products:*

- The antiproliferative and therapeutic effect of *Cotinus coggygia* extracts in carcinoma cases in experimental animals was established.
- After in vitro analysis of the antineoplastic potential of hemocyanins isolated from *Rapana venosa*, *Helix aspersa* and *Helix lucorum* - (HaH-common; HlH-common) and their structural subunits (β c-HaH; α -HaH; β c-HlH; α -HlH), mucus from *Helix aspersa* in various tumor cell lines and their effects on cell viability and proliferative activity, significant antitumor effects of the α -HaH and β c-HlH subunits were found. The results of these studies could contribute to the development of more effective therapeutic agents for the treatment of colorectal cancer.
- It was found that the carotenoids (astaxanthin) from *Coelastrella* sp. BGV microalgae have antiproliferative activity and cause apoptotic cell death in HeLa cells in vitro and a potential antineoplastic effect.
- Significant antitumor effect of synthetic alkylphosphocholine erufosine on Grafi myeloid tumor was found, characterized by reduced transplantation, inhibition of tumor growth, inhibition of metastatic activity and prolongation of the average survival time of tumor-bearing hamsters.
- Studies on newly synthesized multifunctional hydrogels for the treatment of chronic wounds of thiolated hyaluronic acid (HA-SH) and bioactive silver-lignin nanoparticles (Ag @ Lig NPs) show that in a diabetic mouse model these gels lead to complete tissue remodeling and recovery of the skin integrity. These effects of hydrogels define them as reliable substances of treating chronic wounds.

The results of clinical, hematological and histological examinations of the excretory system show that the nano-functionalization of hybrid-coated catheters involving zinc oxide nanoparticles bound to the enzyme amylase is an important step towards the prevention and control of catheter-related infections in the clinic.

- *Study of pathological and clinical aspects of diseases in humans and animals of infectious nature*

- Light microscopic and electron microscopic study of the role of human herpesvirus-6 (HHV-6) in the development of autoimmune thyroiditis (AIT). The possible involvement of HHV-6 gene expression encoding immunomodulatory proteins U12 and U51 in the development of AIT and their role in modulating chemokine signaling was considered.
- After performing nPCR to detect the sequence of HHV-6 in DNA samples, RT-PCR with three different HHV-6 gene targets (U79 / 80, U51 and U12), immunofluorescence microscopy for the expression of HHV-6 antigen and

RANTES (Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted) in thyroid tissue, found that HHV-6 may persist in thyrocytes and interact with RANTES. The high expression of this chemokine in the thyroid gland upon activation of HHV-6 probably promotes the development of AIT.

- BLV has been found to infect animals of various species (rabbits and rats treated with whole blood or serum derived from leukemic cows), including infecting human cells in vitro. These results are supported by comparative studies of hematological parameters in BLV infected animals and patients with T-cell lymphoid leukemia
- Some features in the pathogenesis of pneumoenteritis in calves have been identified. After antigenic and viral diagnosis for various pathogens, by Multiscreen Ag ELISA, Bovine Respiratory and Pulmotest Respiratory Tetra 6 ELISA Kit for Antigen Diagnosis (BoHV-1, BVDV, BRSV and BPI-3 Tissue Lysate Sandwich Test), BIOX Dia and Rainbow calf scour 5 BIO K 306 for detection of Bovine Rotavirus, Coronavirus, Escherichia coli F5, Cryptosporidium parvum and Clostridium perfringens in the faeces of 20% of animals with pneumoenteritis was identified single coronavirus infection, and in the remaining 80% of cases - co-infection with other of mentioned pathogens was detected.
- An important contribution is the detection of parvovirus B19 (B19) in epithelial HOS TE85 cells, as viral genomic DNA is detected in HOS cells during 3-11 passages. Even in infected cells, B19 VP1 and NS1 mRNA expression and the presence of VP1 / VP2 proteins were detected. At high doses (5000-8000 viral genomes / cell) on day 6 after infection, the authors reported massive cell death, with electron microscopy showing that cytopathic effects were associated with apoptosis in HOS.
- Valuable data on the clinical and pathological aspects of acute bacterial infections of exogenous and endogenous origin in exotic animals - rodents and birds
- The distribution of sialylated glycoprotein expression in cell compartments during the muscle trichinosis phase was determined using electron microscopy (labeling with gold nanoparticles conjugated to lectins), histochemical (lectin histochemistry of paraffin sections) and immunohistochemical methods.
- A number of parasitological, clinical and pathomorphological studies of various parasitosis (*Metastrongylus* spp., *Ascarops strongylina*, *Macracanthorhynchus hirudinaceus*, genus *Passalurus*, genus *Nematodirus* and protozoa of the genus *Eimeria* represented by *E. and Magfo*, *E. Media* and *E.perforans*) in domestic animals and game.

- *Study of pathological and clinical aspects of diseases in humans and animals of non-infectious nature:*
 - Pathomorphological and immunological studies in chickens experimentally treated with fumonisin B1, where Dr. Katerina Dimitrova skillfully uses ultrastructural and immunocytochemical methods to determine both ultrastructural changes in important immune organs and intracellular localization of fumonisin B1 in DEC 99 cells. Precisely performed clinical, immunological and pathoanatomical studies allow to make an detailed pathological characterization of fumonisintoxicosis in birds. An important contribution is the established cytopathic effects of FB1 and DON (by quantitative and qualitative methods) on normal and tumor cells: BALB / c 3T3, DEC 99, MDA-MB-231, MCF-7 and Hela as well as the intracellular localization of FB1 has been established by immunogold labeling and transmission electron microscopy.
 - The histological changes in guinea pigs treated with the norsesquiterpene glycoside ptaquiloside isolated from the plant (*Pteridium aquilinum* L. Kuhn), which is considered to be the main etiological agent, were analysed.
 - Lobular and ductal carcinomas of the mammary gland in cats, dogs and humans have been comparatively studied and a case of recurrent post-vaccine fibrosarcoma in cats has been reported. The authors describe in detail the factors and mechanisms responsible for the initiation and development of neoplastic processes and their morphological characteristics.

It is important to note that the candidate applied the wide range of mastered methods, including molecular biological, biochemical, immunological, cellular biological, virological, histopathological and morphological methods for research and interpretation of experimental or clinical cases, at different levels from macrostructural to ultrastructural level and nanoparticle level as well.

As a part of the professional development, Dr Katerina Dimitrova applies a document certifying 170 hours of teaching activity in the form of training courses, as well as assistance in preparing a dissertation or thesis and providing information about the topics in the field of biomedicine of students from different schools, graduates, PhD students and scientists at University of Forestry, Thrakia University, Food Safety Agency, students and PhD students of Rīga Stradiņš University, Latvia.

Dr Katerina Dimitrova has fulfilled and exceeded many of the required criteria of IEMPAM - BAS for holding the academic position "Associate Professor":

Group of indicators	Content	Assoc. Professor	Chief Assistant Professor Dr. Katerina Dimitrova
A	Indicator 1	50	50
B	Indicator 4	100	100.5
Г		200	231
Д		50	80
Sum of all indicators		400	461.5

III. Critical remarks and recommendations

I have no remarks on the presented scientific papers and materials.

Chief Assistant Dr. Katerina Dimitrova is an well-established researcher conducting detailed studies, with sustainable career development and with original contributions to pathology.

IV. Conclusion

I believe that the career development of Chief Assistant Professor Katerina Dimitrova, PhD, her scientometric indicators, the contributions of her research activities fully comply with the requirements of the Law for Development of Academic Staff in the Republic of Bulgaria and in the Regulations of IEMPAM - BAS, Sofia regarding the academic position "Associate Professor".

According to the materials presented to me in the competition, I find that the candidate Dr. Katerina Dimitrova is a specialist with significant original fundamental and practical applied scientific results and contributions. Her studies received international recognition contribute to a more detailed understanding of pathogenesis in different fields of pathology.

I confidently give my positive assessment and recommend the members of the esteemed wacademic position "Associate Professor" in scientific specialty "Animal Pathology", in the field of Higher education 6. Agricultural sciences and veterinary medicine, professional field 6.4. Veterinary Medicine, " at Department of Pathology, IEMPAM - BAS, Sofia.

Prepared the review:

/Prof. Dr. Ivaylo Stefanov, PhD/

18.04.2022r.