To the Chairlady of the Scientific Jury

Order № RD-09-29 / 21.05.2021

of the Director of IEMPAM at BAS

Sofia

REVIEW

By competition for the academic position "Associate Professor" in scientific field 4.3. Biological sciences, specialty "Parasitology and helminthology" (01.06.19) for the needs of the section "Experimental parasitology" at the Institute of Experimental Morphology, Pathology and Anthropology with a museum (IEMPAM), announced in SG no. 30 / 13.04.2021 with the only candidate Ch. Assistant Professor Dr. Ivelin Angelov Vladov.

Prepared by the Prof. Hristo Miladinov Najdenski, 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 are no conditions for conflict of interest between me and the candidate Dr. Ivelin Angelov Vladov 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.

1. **Short biographical data and characteristics of the scientific interests of the candidate**. The candidate for the academic position "Associate Professor" - Ch. Assis. Prof. Dr. Ivelin Vladov graduated from the Faculty of Biology at the Sofia University "St. Kliment Ohridski ", and in 2004 he obtained the Bachelor's degree in Ecology and Environmental Protection, and in 2005 the Master's degree in the same specialty after defending with excellent success a diploma thesis on "Influence of some ecological factors on the dynamics of zooplankton in Boyana Lake". Driven by

his curiosity and interests in the field of experimental biology, he continues his research activity already as a PhD student in independent training at IEMPAM-BAS (Order No. 60/29.01.2014). After successfully defending his dissertation (October 17, 2016) on "Molecular and clinical studies of parasites of the genus Eimeria in an experimental model in rabbits", he received Educational and Scientific Degree "Doctor" in Parasitology and Helminthology. Since 2008 Dr. Vladov has been working as an assistant, and since 2017 he has been a chief assistant at the Experimental Parasitology Section at the new established (by merging) institute - IEMPAM at BAS.

Dr. Vladov's research interests are mainly in the field of biology and pathogenesis of a number of economically significant parasites such as eimeriosis, trichinelosis, fasciolosis, etc., the development of molecular biological, cell culture and experimental animal models for their study, diagnosis and prevention. Certain interests are also found in the field of toxicology, ecology and biochemistry related to intoxications with heavy metals and other natural pollutants. These brief data on the developed thematic areas definitely define Dr. Vladov as a promising researcher in these areas, and his participation in the competition announced by IEMPAM - fully justified and deserved. The accumulated 14 years of scientific experience, the many mastered research methods and personal characteristics are a reliable basis for developing future larger projects and creating successful teams.

2. General description and evaluation of the presented scientific production. Dr. Vladov participated in the current competition with a total of 93 scientific publications published in the period 2008-2021, of which 20 were in foreign and Bulgarian journals with impact factor or impact rank, and 6 in publications indexed in WOS or Scopus, but without IF or SJR. 67 articles are published in non-indexed publications - journals and proceedings of national and international scientific events. I consider the fact that 10 of the total number of publications are in the

leading quartile Q1, 6 in Q2, etc., to be very positive. At the same time, there is no lack of articles published in scientific journals in Bulgarian, which is important for raising the role of Bulgarian scientific periodic issues for the development of science and higher education in our country.

3. Evaluation of fundamental and applied research. The long-term research work of the candidate covers several areas, one of which is experimental toxicology. Advances in this field are crucial for modern clinical medicine and lead to the solution of problems important for human and animal health, as well as for the environment and society as a whole. In a series of publications and with the most modern methods, the accumulation of cobalt and iron in the brain was determined, as well as the changes in the expression of iron-regulating proteins - transferrin receptor 1, hepcidin and ferroportin in mammals. The results show that loading mice with cobalt chloride by drinking water during the late prenatal and early postnatal periods affects iron homeostasis through changes in key Fe-regulatory proteins. In addition, they contribute to the elucidation of the neurotoxic potential of cobalt and the associated health risks in neonates and infants (№ 1).

Original results were also obtained by studying the effect of acute intoxication with sodium nitrite ($NaNO_2$, known preservative in the food industry as E250) on the content of the basic metals iron (Fe), calcium (Ca) and zinc (Zn) in the spleen of mice. Acute $NaNO_2$ intoxication has been shown to lead to changes in endogenous levels of Fe, Ca and Zn in the spleen of mice used as an experimental model. These findings suggest an ionic imbalance and an effect on the activity of antioxidant protective enzymes. This should be taken into account in patients with $NaNO_2$ intoxication to apply appropriate therapeutic approaches and avoid the development of oxidative stress (№ 2).

Research on lead is also important from a toxicological point of view. It is known that humans can ingest Pb by inhalation or ingestion, with acute and chronic exposure to Pb causing central nervous system dysfunction in children and peripheral nervous system damage in adults. Exposure to lead from the environment damages the lungs and is a risk factor for death from cardiovascular disease. The mechanism by which Pb causes toxicity involves a change in the homeostasis of the main elements - calcium, copper, iron and zinc in the lungs and heart. By atomic absorption analysis, it was shown that exposure of mice to Pb for 14 days significantly increased the concentration of toxic metal in both organs and increased cardiac concentrations of Ca, Cu and Fe compared to untreated mice. Pb exposure reduced pulmonary Ca and Zn concentrations compared to untreated controls. In addition, meso-2,3-dimercaptosuccinic acid (DMSA), monensin and salinomycin reduce the concentration of Pb in the lungs and heart. Among the chelating agents tested, only salinomycin restored cardiac Fe concentrations to normal control values. These results indicate the potential use of the polyether ionophore antibiotic salinomycin as an antidote for the treatment of Pb-induced toxicity in the lung and heart (№ 3). The effect of the tetraethylammonium salt of salinomic acid (Sal) on cadmium (Cd) -induced hepatotoxicity and renal dysfunction in Cd-treated mice compared with those treated with DMSA was also studied. Treatment of mice exposed to Cd with DMSA or Sal has been shown to restore the levels of renal and hepatic functional markers and significantly reduce the concentration of toxic metal ions in both organs. Sal administration improves Cd-induced changes in endogenous levels of basic metal ions. Histological examination showed that the antibiotic was more effective in overcoming the toxic effect of Cd on liver morphology than DMSA. Taken together, the results confirm that the anticancer agent salinomycin is a promising antidote against Cd poisoning (№ 5). In these and other studies, the influence of various metal salts (natural contaminants,

food additives, drugs) and their bioaccumulation in various organs under sub-acute, acute and chronic treatment in various experimental in vivo models of rodents, which were skillfully developed and used by the candidate (№№ 5, 9, 45, 46, 60, 91, 94, 96).

In order to monitor the changes in important haematological parameters, an experimental model of acute chemical hypoxia in adult Wistar and ICR mice was successfully developed. This model allows to prove significant morphological and hemorheological changes in the formed elements of the blood under various host effects such as anemia, expressed in a decrease in the number of erythrocytes, the amount of hemoglobin and hematocrit, the number of platelets and a decrease in their volume, which has effect on blood clotting. These indicators can be successfully used as biomarkers for early assessment of the side effects from the application of hypoxia-inducing food supplements and contribute to improving the physical endurance of humans and animals (№ 2, 46).

An important contribution to elucidating the pathogenesis of parasitology diseases are the proven disorders in antioxidant protection and the appearance of micronutrient deficiencies of nutrients (Fe, Se, Cu and Zn) in the serum of experimentally infested animals (№ 13, 16, 48, 62, 68, 69, 70, 91, 96). Based on the proven micronutrient imbalance, appropriate micronutrient formulas are applied to compensate for the host deficiency, which is important for clinical veterinary medicine and practice.

It should be noted with satisfaction that the research started in PhD studies in the field of molecular methods for the diagnosis of parasitological diseases has been successfully continued and is finding more and more practical application. For example, the reference diagnostic techniques used to detect the presence of *Trichinella* in meat are based on direct observation of parasites by microscopic techniques, which, however, are high quality and low sensitivity (1 to 3 larvae per

gram of infected tissue are detected). In contrast, first-time real-time PCR (qPCR) is a fast, highly specific, and sensitive method that allows reliable identification of *Trichinella* infections within 2-3 hours, including species. qPCR has become an important technique in many fields of science, including both in the food industry and in a powerful tool for food quality and safety control. The developed methodology allows adequate control of meat in order to prevent human trichinelosis and as a factor to increase user confidence and protect the health of the consumer. The protocols of conventional PCR and qPCR for examination of other parasitic diseases such as eimeriosis, fasciolosis, hemonchosis, etc. have been optimized, which complements the successfully applied classical methods (№ 11, 15, 25, 38, 50, 75, 80).

The comparative studies on haematological changes in experimentally induced infections with *Trichinella spiralis* and *T. pseudospiralis* in mice after treatment with hemocyanins (macromolecules of natural origin isolated from molluscs) are also original. It has been shown that hemocyanins can successfully perform the function of non-specific and active stimulators of the immune system, improving the body's defenses (№ 79).

In vitro studies on the antitumor activity of biologically active substances of animal origin are also very promising. In addition to the already known immunostimulatory properties, the antitumor activity of hemocyanins isolated from the sea snail *Rapana venosa* and the garden snails *Helix lucorum* and *Helix aspersa*, as well as the mucus from *H. aspersa* snails, was evaluated. Studies on the effects of hemocyanins and mucus on cell viability and proliferation of the HT-29 cell line from human colorectal cancer show that the mucus and α-subunit of *H. snake* hemocyanin have the most pronounced antiproliferative activity, making these substances potential candidates for the development of new therapeutic agents for the treatment of colorectal cancer. Precise cytomorphological analysis performed by

fluorescence and transmission electron microscopy showed that the observed antitumor effects were associated with the induction of apoptosis in tumor cells (№ 7).

A number of publications (№№ 29, 36, 42, 52, 63, 64, 86) reflect the results of hematological, biochemical and histological studies related to development of the biological response in the implantation of new biomaterials with improved physicochemical characteristics, biocompatibility and biological activity. They can be used as implants for bone reconstruction with applicability in veterinary, human and dental medicine.

4. Reflection of the scientific activity of the candidate in the Bulgarian and foreign literature. The active research activity of Dr. Vladov and the mentioned contributions in separate scientific fields of biomedicine have not only constant relevance for the medical and veterinary science, but also open new perspectives for practical application in the diagnosis, prevention and therapy of parasitic diseases, intoxications, microelementosis, osteoinduction and other physiological and pathological clinical indicators in humans and animals. Together with the developed experimental models for their study, these works have found a wide response among the scientific community at home and abroad - a total of 81 citations were observed, mostly by foreign authors and in publications from journals with impact factor.

5. Participation in the implementation of research projects and contractual tasks.
Dr. Vladov's research activity in various research projects is active and fruitful - one under the National Scientific Program "Innovative low-toxic biologically active agents for precision medicine" (BioActiveMed) supported by the Ministry of Education and Science, 8 funded by National Science Foundation, 3 projects under

inter-academic contracts and agreements, 3 under Operative Program "Development of Human Resources ", and 6 projects with the Sofia University "St. Kliment Ohridski ”.

CONCLUSION: The presented scientific papers clearly outline the candidate's participation in the development and implementation of important research tasks, mainly of biomedical significance and in particular with parasitological, toxicological, diagnostic and clinical orientation. Good mastery and use of classical and modern parasitological, molecular biological, morphological and biochemical methods allow obtaining of objective results and precise analyzes, thanks to which significant for human and veterinary medicine scientific and practical contributions are made, most of which are original and reported for first time in the literature. The proven research skills of Dr. Vladov are a reliable basis and guarantee for successful research in these areas and in the future. Completely covering and even exceeding the minimum requirements of the Law on the Development of the Academic Staff in the Republic of 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 respected scientific jury to award Ch. Assist. Prof. Dr. Ivelin Angelov Vladov the academic position "Associate Professor" in scientific field 4.3. Biological sciences and scientific specialty "Parasitology and helminthology".

12.08.2021 Signature: