

To the Chairman of the Scientific Jury
Nominated by order No. RD-15-55/01.11.2024
of the Director of IEMPAM-BAS
Sofia

REVIEW

On the competition for the academic position of "Associate Professor" in the field 6. Agrarian Sciences and Veterinary Medicine, professional field 6.4. Veterinary Medicine, specialty "Parasitology and Invasive Diseases of Animals and Humans" for the needs of the "Experimental Parasitology" section at the Institute of Experimental Morphology, Pathology and Anthropology with a Museum (IEMPAM), announced in the State Newspaper No. 85/08.10.2024 with the sole candidate, Chief Assistant Professor Dr. Delka Salkova Salkova.

Prepared by: Prof. Hristo Miladinov Najdenski, PhD, from the Stephan Angeloff Institute of Microbiology (Bulgarian Academy of Sciences) with expertise in infectious microbiology, molecular biology, immunology, epidemiology, experimental animal models.

I declare that there are no conditions for a conflict of interest between me and the candidate, Chief Assist. Prof. Dr. Delka Salkova Salkova within the meaning of paragraph 1, points 2a, 3, 4 and 5 of the Additional Provisions of the Law for Development of the Scientific Staff in the Republic of Bulgaria (LDSSRB). The documents provided to me for the competition for preparing a review have been correctly prepared and comply with the LDSSRB and the Regulations for its application in IEMPAM-BAS.

1. Short biographical data and characteristics of the candidate's scientific interests. The candidate for the academic position of "Associate Professor" Dr. Delka Salkova Salkova was born in 1976 and graduated in 2000 from the Faculty of Veterinary Medicine of Thracian University - Stara Zagora with the educational and qualification degree "Master" and professional qualification "Veterinary doctor". In 2005 she successfully defended a PhD thesis on the topic

"Influence of NH_4VO_3 on the development of the nematode *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 and host tomato" and acquired the educational and scientific degree "Doctor" (PhD).

In the following years, Dr. Salkova built on her research experience and knowledge on molecular methods for the diagnosis of current parasitosis at the population and species level and modern bioinformatics approaches for processing nucleic and protein sequences, in parallel with the application of classical microscopic and biological techniques for species determination of nematodes in plants and animals. A very positive effect on her professional development was also exerted by the specialization carried out in Russia and her participation in numerous specialized courses, which enriched her professional skills and competencies.

All these briefly presented biographical data testify to the formation of lasting scientific interests in current biomedical directions and clearly outline the profile of Dr. Salkova as a promising researcher in the field of clinical and experimental parasitology.

2. General description of the submitted scientific production. In this competition, the candidate presents a total of 57 scientific papers, of which one is an Abstract for obtaining the PhD degree and 56 publications in specialized scientific journals, which are distributed as follows: scientific articles in journals indexed in WoS or Scopus in total 11, articles and reports from international and national scientific forums published in journals referenced in world-renowned databases in total 14 and articles and reports published in non-refereed journals with scientific review - 31. Particularly impressive are the publications in the journals Veterinary Sciences (Q1), Diversity (Q1), Bulletin of Entomological Research (Cambridge University Press, Q1), etc. Dr. Salkova's participation in the submitted scientific articles is emphasized as leading author - in 24 she is the first author, in 7 she is the second author, in 7 she is the third author, and so on. To the scientific assets should be added the serious participation of the candidate with a total of 57 presentations at scientific forums, 17 of which abroad and 40 scientific forums held in Bulgaria, most of which with international participation.

3. Evaluation of scientific research and scientific applied activity. The overall research work of Dr. Delka Salkova is focused on several thematic areas related to the study of bee pathology, current parasitosis in domestic and wild animals and some modern genetic and molecular-phylogenetic studies in local animal breeds. The object of intensive research is also the influence of various vanadium glycosides and radiation exposure on the biological cycle of plant parasitic gall nematodes. Studies on new combinations of essential oils and plant extracts on the health status of bee colonies, the effectiveness of various antiparasitic agents in domestic and wild animals, the presence of resistance to some veterinary medicinal preparations introduced for use in practice, etc. are with a greater practical focus.

Dr. Salkova's research on a number of current issues related to the breeding of honey bees and their role as bioindicators of environmental pollution with pesticides and heavy metals is very in-depth. In this regard, for the first time in our country, an analysis of DNA isolated from honey samples originating from different regions of the country was conducted. The presence of pathogenic microsporidia of the species *Nosema apis* and *Nosema ceranae* and the parasite *Varroa destructor* was proven (publ. B5). Similar studies on RNA isolated from bee products (pollen, royal jelly, etc.) prove the presence of the most widespread viruses among bee colonies - deformed wing virus (DWV), sacbrood virus (SBV), acute blight virus (ABPV) and chronic bee paralysis virus (CBPV), etc. The phylogenetic analysis allows to clarify not only the origin, but also the genetic diversity of the Bulgarian isolates in comparison with the homologous sequences available in the genetic database. These studies outline the future potential of this approach for biomonitoring of current infectious diseases in bees (publ. B1, B3, B5).

For the first time in our country, the species diversity of the bee microbiome has been studied in families from different geographical regions of the country. Using a reliable molecular method (sequencing of the 16S rRNA gene), the main genera of bacteria inhabiting the intestinal microbiome of bees have been identified, namely: *Bartonella*, *Bifidobacterium*, *Snodgrassella*, *Frishella*, *Gilliamella*, *Lactobacillus* and *Commensalibacter* (publ. B4). An original study on the pathogenesis of nosematosis in bees (conducted jointly with scientists from the Estonian Academy of Sciences) proves that *Nosema apis* alone or in mixed invasion with *Nosema ceranae* causes

interruption of metamorphosis at different stages of the development of the queen bee, negatively affecting the process of her hatching (publ. B6). Additional data on the distribution of these two pathogens show that as a result of climate change, *Nosema ceranae* is becoming a dominant species in countries with a traditionally cold climate, which should be taken into account when diagnosing the increased cases of latent nosematosis (G2, G17). In this direction, studies on the distribution of *Varroa destructor* - an ectoparasite that causes huge economic losses to beekeeping, as well as its role as a vector for the spread of a number of viral diseases are also very relevant (publ. G19, G27). It has been established that 1/3 of the samples studied also show mixed infection by the two pathogens - *Varroa destructor* and *Nosema ceranae* (G5, G16, G18, G21, G25, G26, G31, G35). These studies, the results obtained and the competent analysis of the multitude of literature data are important in order to prepare recommendations for the prevention and control of nosematosis and varroatosis.

A second important focus in Dr. Salkova's scientific research is the spread, diagnosis, treatment and prevention of current parasitosis in wild and domestic animals. Original data reveal the potential role of red foxes and the ticks they carry in the epidemiology of rickettsioses in humans and animals, proving for the first time in our country the presence of bacteria of the genus *Ehrlichia* in ticks of the species *Rhipicephalus sanguineus*, as well as rickettsiae in ticks of the species *Ixodes ricinus* (publ. B8, B9).

The observed unfavorable trend towards an increase in the spread of heartworms in Bulgaria is a reason for conducting a screening microfilaremia study of canids from different regions of Bulgaria. Morphological differentiation not only identifies the species *Dirofilaria immitis*, *D. repens*, *Acanthocheilonema (Dipetalonema) dracunculoides* and *A. reconditum* as some of the most common causes of microfilaremia in canids (the first two with zoonotic potential), but also contributes to updating the epizootological data regarding dirofilariasis in our country and emphasizes the need for constant control of the disease among canids (G34). A group of studies are aimed at supporting the hypothesis of suppression of carcinogenesis in the liver by biologically active substances isolated from *Fasciola hepatica* L. tissue and from infected spleen in rats. An inhibitory effect on cell

proliferation has been proven and an analysis of the data on the relationship between different combinations of trematodoses and the development of tumors in animals and humans has been made (G29, G32, G33, G40, G41).

A series of laboratory studies have demonstrated the adaptogenic effect of furostanol glycosides extracted from the plant *Dioscorea deltoidea* on the content of trace elements and the development of tomato plants invaded by the nematode *Meloidogyne arenaria*. The results obtained from atomic absorption spectrometry show a tendency towards normalization of the content of the trace elements Zn, Cu, Fe and Mn, and can serve to build a new strategy for compensating of oxidative stress in invaded plants (publ. G9, G20, G43). In search of new means for combating root-knot nematodes, different concentrations of ammonium vanadate have been studied. The observed changes in the mineral composition, as well as electron microscopic changes, prove that the optimal treatment dose is 0.01 mg, and the concentration of 0.13 mg is the highest non-toxic dose, favorably affecting the development of the root system (G11-14, G45, G46).

Interesting data on the domestication of the horse, and especially on the Arabian horse as one of the oldest breeds out of over 500 known horse breeds in the world, are presented in publication G1. For the first time in Bulgaria, the population structure and genetic diversity among the existing paternal lines of Arabian horses in Bulgaria have been studied, using 15 microsatellite markers. The study, including 537 Arabian horses, shows high heterozygosity, with the LATIF and SAKLAWI I lines showing the highest values (up to 0.67), and the inbreeding coefficient for the entire Arabian population is quite low: up to -0.041 in SAKLAWI I. Other similar studies on goats (Kalofer Longhair and Bulgarian Vitoroga Longhair), as well as on three Bulgarian mountain sheep breeds (Rhodope Tsigai, Middle Rhodope Sheep and Karakachan Sheep) would contribute to the development of a national strategy for excluding non-purebred animals from breeding programs in order to preserve the genetic profile of the original studied breeds.

The skills and research experience accumulated by Dr. Salkova are the basis of some studies with a practical focus. The veterinary preparations "Varostop" (containing flumethrin and an analogue of Bayvarol), "Ecostop" and "Mentotim" (based on thymol and mint oil, respectively) were studied for resistance and

effectiveness against the mite *Varroa destructor*. The authors conclude that due to its high effectiveness (over 90%, and in some cases up to 99%), the lack of harmful effects on bee colonies and the lack of resistance make flumethrin a suitable acaricide for the control and prevention of varroa disease in Bulgaria and can meet the needs of beekeeping practice.

In response to the demand and creation of new quality food supplies for bee colonies in early autumn, the influence of the natural plant extract IMMUNOSTART HERB on the strength of the bee population, the amount of pollen obtained, the productivity of the queen bee and the yield of honey was monitored.

The results obtained show that the applied nutritional supplement positively affects all studied biological parameters, with the most noticeable effect after the second application and emphasize the potential of herbal supplements for effective development of bee colonies during the period of shortage of bee pasture, as well as providing appropriate conditions for successful wintering.

A series of literature reviews have analyzed numerous challenges facing managers in hunting farms and veterinary specialists regarding the use of antiparasitic agents in wild boar, mouflon and ruminants of the *Cervidae* family (publ. G30, G23, G22). Not only the individual groups of antiparasitic agents and their features have been competently analyzed, but also the specifics related to their application in wild animals raised in their natural habitats.

4. Reflection of the candidate's scientific activity in Bulgarian and foreign literature. The candidate's versatile and active scientific research activity on current issues in the field of veterinary medicine has found a response among the scientific community in our country and abroad. The candidate's papers have been cited 39 times, which testifies to the demonstrated research interest in the published results. It should be noted that the articles are cited mainly by foreign authors (37 citations) and in publications from prestigious international journals with a high impact factor.

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

Dr. Salkova's active research activity and high professionalism also determine her fruitful work on a total of 12 scientific projects, in 3 of which she is the head of working groups, and in 9 she is a participant. An important role for her scientific development and growth is played by the leadership of two projects at the National Diagnostic Research Veterinary Medicine Institute and one at the bilateral interacademic contracts with the Estonian Academy of Sciences. Her participation in many other projects on bilateral international cooperation within the framework of interacademic contracts and agreements of the Bulgarian Academy of Sciences with the Russian Academy of Sciences, the Slovak Academy of Sciences, the Agricultural Academy and the National Research Fund is also a key factor. She has also successfully completed research tasks set by individual trade companies.

CONCLUSION: The overall long-term research activity of Dr. Salkova is aimed at solving important issues for veterinary medicine science and practice. It marks an upward development, connecting with the mastering and application of a wide range of classical and modern molecular biology, genetic, morphological, microscopic, etc. methods. The comprehensive scientific research activity presented by the candidate is not only significant and relevant, but also a reliable platform for increasing the scientific capacity, diagnostic and expert potential of IEMPAM. The experience gained, the active and fruitful work in the above-mentioned areas will undoubtedly contribute both to the more effective development of beekeeping and some other branches of animal husbandry, and to the therapy and prevention of the above-mentioned infectious diseases and parasitosis in animals and plants. The proven research skills of Dr. Salkova are a reliable platform and guarantee for successful scientific research activity in the field of biomedicine in the future. Fully covering the minimum requirements of the LDSSRB, as well as the required criteria of the IEMPAM at the Bulgarian Academy of Sciences, and considering all the arguments mentioned above, I propose to the esteemed scientific Jury to award Chief Assistant Professor Dr. Delka Salkova Salkova the academic position of "Associate Professor" in the professional field 6.4. Veterinary Medicine, specialty

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03.02.2025 г.

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