

REVIEW

by Prof. Ivan Dinev Ivanov, Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora

Member of the Scientific Jury on the basis of Order №3ПЦ – 173/11.05.2020 of the Rector of University of Forestry, Sofia, on participation in:

competition for the academic position "Professor" in the scientific specialty Animal Pathology, in the discipline "Pathology /Special Pathological Anatomy/", field of higher education 6. Agricultural sciences and veterinary medicine, professional field 6.4. Veterinary medicine, published in the State Gazette, issue 32 of 03 April 2020 and procedure code VM-P-0320-35.

Candidate in the announced competition is Assoc. prof. Dr. Vasil Kostadinov Manov from the Department of Internal Noninfectious Diseases, Pathology and Pharmacology of the Faculty of Veterinary Medicine at the University of Forestry, Sofia.

• **Brief biographical data.** Assoc. prof. Dr. Vasil Kostadinov Manov was born on December 5, 1962 in the village of Divlyia, district Sofia. Graduated in veterinary medicine at the Higher Institute of Zootechnics and Veterinary Medicine (HIZVM), Stara Zagora in 1989. After several months of experience as a veterinarian responsible for infertility and neonatal mortality at PPMM "Road to Communism", Byala Slatina, in 1989 Assoc. prof. Manov won a competition for a full-time assistant in the then Department of "Pathological Anatomy" at the Faculty of Veterinary Medicine at HIZVM, Stara Zagora, where his development continued as a senior assistant (1993) and chief assistant (1996). From the autumn semester of the same year he moved to work at the recently opened Faculty of Veterinary Medicine (FVM) at the University of Forestry (UF), Sofia.

In 2009 he successfully defended a dissertation on "Comparative pathomorphological studies in animals infected with Bulgarian isolates of Aujeszky's disease virus" for which he was awarded the Educational and Scientific Degree (ESD) "Doctor". Since 2010 he has held the academic position of Associate Professor at the Department of Internal Non-Communicable

Diseases, Pathology and Pharmacology of FVM at UF, Sofia. The biographical information of the candidate also includes a six-month course for studying English in a postgraduate qualification and language proficiency course at level B1, proficiency in Russian at level B2 and membership in a medical commission at the BFSA, a legislative commission at the Ministry of Agriculture, Bulgarian Association of Veterinarians for Small Animals and Union of Veterinarians in Bulgaria.

• **Description of the materials for participation in the competition.** According to the rules for the development of the academic staff of the University of Forestry, Sofia, the documents and materials received by me for the preparation of a review fully comply with the requirements.

Assoc. prof. Manov presents a list and copies of materials certifying the existence of a total of 82 scientific papers (including dissertation and abstract). Of these, 46 for participation in the announced competition are indicated, of which 37 publications, 2 monographs, 2 textbooks and 5 tutorial manuals for students. Of course, all normative documents are attached to the set of materials according to the requirements (diplomas for completed higher education, for a scientific degree, information for pedagogical and teaching activity, etc.).

• **General characteristics of the candidate's activity.**

Research activity. Apart from the scientific production related to obtaining the ESD "Doctor" and the academic position of associate professor, the works after the previous habilitation are subject to review for participation in the current competition. In connection with this, a list consisting of 37 pieces is presented. scientific publications, grouped and arranged according to the thematic focus.

Publications from №1 to №15 are categorized in the direction "Pathological and pharmacological studies of samples of plant origin". It is worth noting for the candidate that they are all in publications that are referenced and indexed in world-famous databases of scientific information (Scopus and Web of Science). The conducted research reflected in a significant part of these works results in scientific contributions of original character (№№1-3; №5, №№8-13, №15). For the first time, a series of in vitro / in vivo studies for the protective effect of purified extracts, saponin mixtures and biologically active substances obtained from *Gypsophila trichotoma* Wend., Three species of the genus *Astragalus* L. and *Ruscus aculeatus*

L. were performed. the nature and extent of involvement of various organs, which confirms the obtained pharmacological data. In the histopathological examination of the livers of the animals treated with plant samples, pronounced protective effects were observed, manifested by the accumulation of fat in a limited number of cells and the absence of necrotic changes in the hepatocytes. No hemodynamic lesions and degenerative-necrotic changes in ganglion and glial cells were found in the brains of animals treated with a toxic agent and protected with plants.

Studies in publications №№1-3 strongly demonstrate the *in vitro/in vivo* hepatoprotective potential of the flavonoid saponarin isolated from *Gypsophila trichotoma* in models of hepatotoxicity with paracetamol (1) and carbon tetrachloride (2). Hepatoprotective and antioxidant effects have also been found *in vitro/in vivo* for butanol extract of *A. monspessulanus* subsp. *monspessulanus* in CCl₄-induced liver damage commensurate with silymarin activity (3).

The results of the studies included in publications №9 and №15 reveal the established *in vitro/in vivo* protective effects of alcesepholiside isolated from the aerial part of *Astragalus monspessulanus* subsp. *monspesulanus*. Neuroprotective, hepatoprotective and antioxidant activity comparable to silibin/silymarin has also been demonstrated in non-enzymatic lipid peroxidation with Fe²⁺/AA and in a CCl₄-induced brain and liver toxicity model.

Research in this area is complemented by evaluating the hepatoprotective activity under conditions of t-BuOOH-induced oxidative stress, as well as neuroprotective - in a model of damage with 6-OHDA, comparable to that of silibin proven for flavoalkaloids and flavonoids isolated from *A monspesulanus* subsp. (11). Hepatoprotective and neuroprotective effects were found *in vitro* on a purified saponin mixture derived from *Astragalus glycyphylloides*, alone for toxicity and in models of t-BuOOH intoxication (8 and 13).

In another study in this field, the antioxidant potential of skimmed extract of *Astragalus spruneri* in spontaneously hypertensive rats (SHR) was found, emphasizing that compared to normotensive animals, the extract affects the activity of antioxidant enzymes in the liver, kidneys and spleen. (5). Results have been published that purified *Ruscus aculeatus* extract (ERA) containing 20% steroidal saponins showed effects on the bone structure of rats with estrogen deficiency induced by bilateral ovariectomy. It has been suggested that ERA may be a potential candidate for the prevention of postmenopausal osteoporotic complications (12).

The field categorized as "Clinical, pathological and differential diagnostic assessment of some significant for veterinary practice infectious diseases and neoplasms" includes 16

publications. In 13 of them scientific contributions with original and in 3 with confirmatory character are highlighted. The canine parvovirus, established for the first time in our country in samples from wild animals and domestic cats, stands out here. This contribution is the result of a 10-year molecular biological study to prove parvovirus infection in samples obtained from carnivores in Bulgaria. The field strains proven in clinical cases are distinguished from the vaccine strains used for prophylaxis in practice (4).

In publications under numbers 16, 20, 31 and 34 in the same section are published data in connection with current pathological problems of industrial pig breeding. The etiological role of porcine circovirus 2 (PCV2) in the induction of new circovirus-associated diseases for the country has been demonstrated: multisystem weight loss syndrome after weaning (PMWS); dermatitis and nephropathy syndrome in pigs (PDNS); respiratory disease; reproductive disorders as well as general or persistent signs of the disease. PDNS has been described in detail, with depression, ataxia and paresis red-violet spots, mainly in the perineal area and hind limbs, pale kidneys with petechial hemorrhages in the cortex, hemorrhagic lymph nodes and interstitial pneumonia (16 and 20). Comparative pathomorphological studies were performed in neonates not weaned colostrum pigs after infection with a vaccine and two uterotrophic strains of Pseudorabies virus. Histological, histochemical and electron microscopic examinations revealed non-purulent meningoencephalitis, serous-fibrinous pleurisy, fibrinous-necrotic and productive pneumonia and the presence of Cowdry-type intranuclear inclusions. Different tropism and intensity of the changes induced by the different viral strains in the body of pigs were found (31 and 34).

In separate works classified in this direction pathomorphological findings with important differential diagnostic significance have been established. The clinical manifestations and pathomorphological changes caused by a vaccine strain and two field uterotrophic strains of Suid herpes virus 1 in young dogs and cats were studied. One of the field isolates was found to be highly virulent in dogs and cats, causing a fibrinous-necrotizing inflammatory reaction in the lungs of cats. It has also been confirmed that the vaccine strain is more pathogenic in dogs and less pathogenic in cats, and the pathohistological changes are localized in the nervous and respiratory systems and have the character of non-purulent encephalitis and interstitial pneumonia (32). A single case of paratuberculosis in cattle in the country has been described (official statistics in Bulgaria lack accurate data on its prevalence). Pathological changes characteristic of this disease have been identified. On microscopic examination of pathologically altered areas of the small intestine, typical mycobacteria were observed.

Conventional PCR showed the presence of DNA from *Mycobacterium avium* ssp. paratuberculosis (27). On the basis of cytological and pathohistological examination, hepatocellular carcinoma, liver necrosis and hepatic jaundice were diagnosed in a 6-year-old female dog of the Caucasian Shepherd breed. The anaplastic and epithelial origin of the tumor formation was confirmed by immunohistochemical examination with polyclonal carcino-embryonic antigen and cytokeratin 7 (19). The neuroendocrine origin of a tumor in a dancing Eurasian brown bear has been proven. An imaging examination of the chest with CT was performed and the following were found: closed, left-sided pneumothorax, atelectasis and displacement of the mediastinum to the right. Macroscopically, prominent, nodular lesions were found in the liver, and in the lungs - atelectasis, anthracosis, pneumosclerosis, tumor-like structures, venous stasis, hemorrhage and mineral deposits. Epithelial cell communities with histological features characteristic of neuroendocrine tumors have been observed in the liver and lungs. The immunohistochemical reaction of TTF-1 to lung tissue is negative, thus ruling out the possibility that it is lung adenocarcinoma. The presented data for a positive reaction with cytokeratin and two neuroendocrine markers give high reliability for the origin of the tumor (21).

Some of the publications in this group present contributions of a confirmatory nature. These include developments that have successfully performed in ovo tests to demonstrate the toxic and carcinogenic potential of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) on turkey embryos (23) and Japanese quail (25), and the result that NDEA induces neoplastic lesions in the liver of White Leghorn chickens (6). The data that in ovo tests are a suitable model system for experimental studies on embryos of White Leghorn hens, line 15I and guinea fowl with chemically induced neoplasia have also been confirmed (6).

No less valuable are the contributions of an applied nature. A differential diagnostic scheme of clinical signs and macroscopic changes in infectious abortions and stillbirths in pigs has been developed to facilitate and guide the actions of the veterinarian (37). A valuable model system for experimental cancer research through hepatocarcinogenicity studies of chemical compounds on avian embryos is presented (6, 23, 25, 29). Conducted pharmacological and pathological studies with biologically active substances of plant origin, proving well-defined protective activity, could be a prerequisite for the development of phytoproducts in the future and their application in human and veterinary practice (1-3; 5, 8-13, 15, 33).

In addition to publications that exceed the required minimum for participation in the competition, the candidate has presented curricula, manuals and aids directly related to his teaching work, individually or in co-authorship. Author's textbooks and scientific monographs are included in this list. The listed textbooks filled a long-standing lack of current ones in FVM at UF, thanks to the initiative and competence of Assoc. Prof. Manov. They are an absolute necessity for the training of students and professionals working in this field.

In the period after his habilitation in 2010, Assoc. Prof. Manov participated in the team of two national projects. He was the research supervisor of a successfully defended doctoral student. He is a member of the editorial board of a scientific journal published by FVM of UF Sofia.

Teaching activity. The direct duties of Assoc. prof. Manov are related to conducting a lecture and practical course in the disciplines "Pathology (general pathomorphology) - 30 lecture hours and Pathology (special pathological anatomy) - 60 lecture hours. Participates in the establishment of laboratories for the preparation of macroscopic preparations and pathohistological examinations at the faculty. He was the head of the University Clinic for Small Animals "Academica" until 2016. A report on the annual classroom employment of 360 teaching hours is presented.

• **Reflection of the candidate's scientific works in the literature.** From the presented reports for scientometric indicators it is evident that from the publication activity the candidate has received a total impact factor of 23,659 from 12 scientific papers published in international journals. In 7 of the publications in connection with the competition he is an independent author, in 4 in co-authorship with 1, respectively 2 and in 35 with more co-authors. Assoc. Prof. Manov presents a list of a total of 86 citations from publications in international publications.

• **Some notes.** Although according to the Regulations for the development of the academic staff at UF it is not specified as a mandatory requirement, the candidate could contribute to the promotion of his work in the country through some publishing activity in popular and popular science magazines, although he has published monographs. I think that in this way the achievements in the field in which he worked will become even more accessible and would satisfy a certain collegial interest. I hope he will be able to compensate for this to a large extent after the completion of the competition procedure.

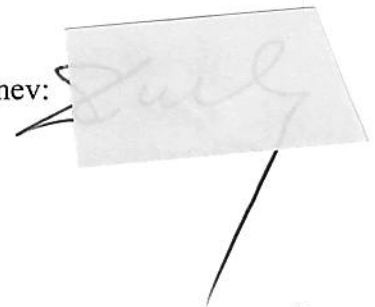
• **Personal impressions.** My acquaintance with Assoc. prof. Vasil Manov dates back to the competition through which we were simultaneously appointed as assistants in the Department of Pathological Anatomy of HIZVM, St. Zagora, at the end of 1989. If I have to describe him personally in one sentence, I would say: this person has the potential to take the academic position for which he is applying. He managed to prove this, especially after moving to FVM at UF, Sofia in 1996. I'm not sure which is the more accurate definition of "co-founder" or "founder" of our related department at this faculty, but in both cases I think that means a lot. Just imagine that it started from scratch, and today it is a place where regular classes can be held in the disciplines we teach. In addition to the basic, Assoc. prof. Manov also contributes to the personnel development necessary to ensure the educational process. He managed to bring out a full-time doctoral student, who today holds the position of chief assistant. It is no exaggeration to say that the value system he has built lacks nothing in terms of morality, ethics and academic behavior. Of course, I could add a lot of clichéd concepts specific to this section here, but I consider this superfluous.

• **Conclusion.** Analyzing the research and teaching activities on the basis of presented scientific productivity, references to reflect the contributions of publishing activity and academic employment of the candidate, I believe that they fully meet the requirements of the law for the development of academic staff in Bulgaria, the rules for its application and the criteria of the Faculty of Veterinary Medicine at the University of Forestry, Sofia for acquiring the relevant position in this competition.

In view of this, I invite the esteemed members of the scientific jury to support my review and join my proposal to the Faculty Council to award the academic title "Professor" in the scientific specialty "Animal Pathology", in the discipline "Pathology /Special Pathological Anatomy/" of Assoc. prof. Dr. Vasil Kostadinov Manov, from the Department of Internal Noninfectious Diseases, Pathology and Pharmacology of the Faculty of Veterinary Medicine at the University of Forestry, Sofia.

July 15, 2020

prof. I. Dinev:

A handwritten signature in black ink, appearing to read "I. Dinev", is written on a rectangular piece of paper. The paper is slightly tilted and has a thin black line extending downwards from its bottom right corner.