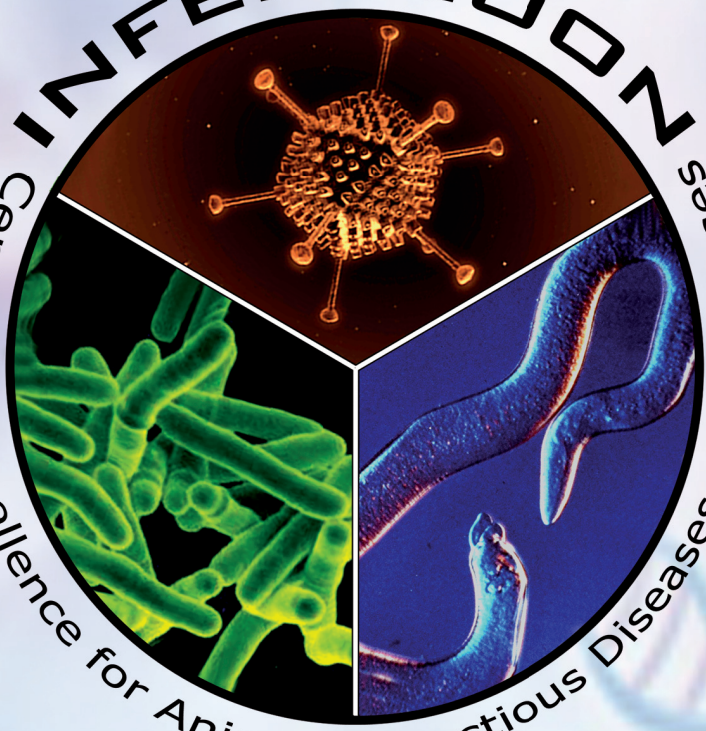
Agentúra
Ministerstva školstva, vedy, výskumu a športu SR
pre štrukturálne fondy EÚ

Európska únia
Európsky fond regionálneho rozvoja

Operačný program
VÝSKUM a VÝVOJ

INFEKTZOON



Center of Excellence for Animal Infectious Diseases and Zoonoses

University of Veterinary Medicine and Pharmacy in Košice

Institute of Parasitology of Slovak Academy of Sciences in Košice

The University of Veterinary Medicine and Pharmacy in Košice through the ages

The University of Veterinary Medicine and Pharmacy in Košice (UVMPh) was established by an Act of the Slovak National Council on 16 December 1949 as the Veterinary College in Košice, though it began its teaching activities with its first lecture on 5 October 1949. Its founding was the culmination of many years of effort aimed at establishing a school of veterinary medicine in Slovakia, since before then those interested in such study had to go abroad.

In 1952, by government resolution, the Veterinary College in Košice lost its independence when it was attached as a veterinary faculty to the Agricultural University in Nitra. Efforts to return it to independence were finally sealed by ratification of a law in 1968, and from the start of the 1969/1970 academic year, it again became an independent university. This was reflected in the change of the school's name in 1992, when the Veterinary College in Košice was renamed as the University of Veterinary Medicine in Košice. With the introduction of a new study program in pharmacy in the 2006/2007 academic year, the university requested another name change, and as of 15 January 2010 it bears a new name: the University of Veterinary Medicine and Pharmacy in Košice.

The study programs with the longest tradition are the **general veterinary medicine** and the **food hygiene** programs, which in the 2006/2007 academic year were expanded with a study program in **pharmacy**. Veterinary medicine and pharmacy rank among the so-called regulated professions, the study of which must meet the demands of European Union guidelines. The UVMPh in Košice meets the standards required by the EU, and a diploma conferred by the university is valid in all countries of the European Union, and that, together with the high level of provided education, attracts students to study veterinary medicine in the English language, particularly from Israel, Sweden, Norway, Ireland, Great Britain, Greece, Cyprus, Iceland, Belgium, Austria and Japan.

In addition to these second degree study programs, the university offers education of the first degree in two study programs – **cynology** and the **safety of feeds and foodstuffs** with two related study programs of the second degree – **production health of animals and environmental protection and the market and quality of foodstuffs**.

The university offers a higher education of the third degree in 16 accredited study programs with a standard length of at 4 years in full-time, or 5 years in the external form of study – **food hygiene; veterinary morphology and physiology; internal diseases of ruminants and swine; internal diseases of horses, small animals and poultry; veterinary surgery, orthopaedia and radiology; veterinary obstetrics and gynecology; infectious diseases of animals; parasitic illnesses of animals; forensic and public veterinary medicine; animal nutrition and dietetics; hygiene of animal breeding and the environment; veterinary biochemistry; microbiology; immunology; virology and toxicology**.

A confirmation of the quality and permanently high level of scientific, research and educational activities of the university is its repeated evaluation by recognized authorities on the national and international levels. UVMPh in Košice was the first university in Slovakia which passed through complete accreditation, and in 2008 it obtained the right to confer the relevant academic titles to graduates of all study programs offered by the university at all three degrees of university study. The university also has acknowledged the right to perform educational activities and activities for designating professors in 12 fields of study – **microbiology; immunology; food hygiene; veterinary morphology and physiology; internal diseases of animals; veterinary surgery; orthopaedia and radiology; veterinary obstetrics and gynecology; infectious and parasitic diseases of animals; forensic and public veterinary medicine; animal nutrition and dietetics; hygiene of animal breeding and the environment; and toxicology**.

In that same year the Slovak Ministry of Education issued to the university, on the basis of the complete accreditation, a certificate of its worthiness to perform research and development. The university was reviewed in three areas and in the fields of life sciences, and in veterinary sciences obtained the highest award – top international quality – and in a third field of chemistry, chemical technology and biotechnology, it obtained an award for internationally acknowledged quality.

On the international level an evaluation of the approximation of education in the field of veterinary medicine between the Slovak Republic and the European Union was performed on the university by the EU Commission-TAIEX, a re-evaluation was done by the Brussels-based European Association Establishments for Veterinary Education (EAEVE), of which UVMPh in Košice is a member, and an institutional evaluation was conducted by the European University Association (EUA).

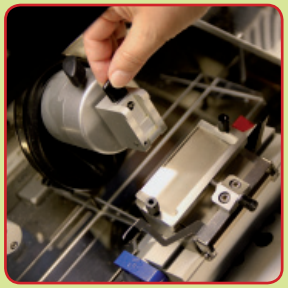
The conclusions of all of these evaluations were favorable for the university, and they confirmed that the university has many strong sides, primarily in the field of its unique orientation on the field of veterinary medicine, food hygiene and pharmacy, and further in the high qualification of the level of academic and technical personnel, the obtaining of revenues from research, the good success of its graduates on the labor market not only in Slovakia but also abroad, as well as the provision of education in the English language for students from abroad and its success in evaluations by external rankings and ratings agencies.

International cooperation

The role of the university in its cooperation with other universities and research institutes both at home and abroad is significant and, in the field of veterinary education from the statewide and international point of view, unchangeable.

Cooperation with other universities is realized in the framework of the university's active membership in the European Association of Establishments for Veterinary Education (EAEVE), the European Veterinary Network of Students Staff Transfer (VetNEST), the Wild Animals Vigilance Euromediterranean Society (WAVES), the Association of Carpathian Region Universities (ACRU) and the Slovak Academic Association for International Cooperation (SAAIC).





Structural funds

UVMPh in Košice, in the scope of drawing on financial resources from the structural funds of the European Union for the program period 2007-2013, became involved in two operational programs: **Research and development** and Education.

In the scope of the operational program **Research and development**, measure 2.1 and the appeal **Support for centers of excellence**, whose goal is to raise the quality of research workplaces and to support excellent research with an emphasis on areas of strategic significance for the further development of the economy and society, co-financed from the European Fund for Regional Development and the state budget of the Slovak Republic, the university was successful in the scope of the project **Center of excellence for animal infectious diseases and zoonoses – INFEKTZOON**.

Another successful project in the scope of this same operational **Research and development** program, measure 5.1 and the appeal **Support of infrastructure for universities for the purpose of improving university conditions**, the university was successful with a project under the name **Increasing the quality of education by expanding and modernizing ICT networks and facilities at the University of Veterinary Medicine in Košice – I. and II**.

In the scope of the operational program **Education**, measure 1.2 and the appeal **Universities as the motors of scientific development of society**, the university was successful with a project under the name **New study programs and education at the University of Veterinary Medicine in Košice**. In the scope of the mentioned project the university is preparing two new study programs for accreditation and instruction, namely a bachelor's program under the name **the human-animal relationship and its use in canis- and hippotherapy,** and a doctoral study program under the name **neurosciences**. In the scope of the mentioned project the university is also preparing and implementing education for university management and for university teachers.

With the creation of projects and applications for them we have also cooperated with other universities, primarily with P. J. Šafárik University in Košice and the Technological University in Košice, as well as with professional institutions associated with the Slovak Academy of Sciences. The result of bilaterally advantageous cooperation are two successful partnership projects in the scope of operational program **Research and development**, measure 2.1 and the appeal **Support for centers of excellence**, or the appeal **Support of networks of excellent workplaces for research and development as pillars of development for the region and support of inter-regional cooperation**.

In partnership with the Parasitological Institute of the Slovak Academy of Sciences (SAS) in Košice we were successful in a project under the name **Center of excellence for parasitology** and in partnership with the Medical Faculty of UPJŠ in Košice, the Institute of Material Research of the SAS, the Technological University in Košice and the Institute of Physiology of Agricultural Animals of the SAS, the university will be a part of the team in a project under the name **Center of excellence for biomedical technology**.

Another result of bilaterally advantageous cooperation with the mentioned institutions in the scope of the operational program **Research and development**, measure 2.2 and of the appeal **Support for applied research, development and transfer of technology**, is a project obtained in partnership with the Medical Faculty of UPJŠ in Košice and the Institute of Material Research of the SAS under the name **Advanced implants with seeded stem cells for the regeneration and reconstruction of hard tissues** and a project obtained in partnership with the Neurobiological Institute of the SAS under the name **The creation and development of a diagnostic approach when treating trauma of a damaged spinal cord**.

The university has for the listed projects thus far received 14,718,415.80 euro from EU structural funds, from which 10,108,953.94 euro have been earmarked for facilities and equipment and 3,126,117.43 euro for construction investments.

Research at the university and the prerequisites for the origin of a center of excellence

The scientific activities of the University of Veterinary Medicine and Pharmacy in Košice are focused primarily on these areas:

- infectious and invasive diseases of agricultural and hobby animals,
- non-infectious diseases of agricultural and hobby animals,
- creation and protection of the environment for animals and people,
- hygiene, production and processing of healthy, non-harmful foodstuffs,
- pharmaceutical research.

Quality results published in international scientific journals means, among other things, collective engagement in the study of infectious diseases of animals. Experimental work in this field of research utilizing the latest molecular-genetic methods had already begun by the 1980s. These methods included, for example, the isolation of DNA, the cutting of DNA with restrictive enzymes, hybridization techniques, the cloning of recombinant DNA molecules in *E. coli* and others. In the 1990s the PCR and RT-PCR methods for the sequencing of nucleic acids were added and at that time proteomic methods began being used.

Although our university ranks among those with the highest success rate for obtaining grants on the national and in part the international level, deeper analysis indicates that research is markedly fragmented. Often small groups applied for grants and these lacked the ambition or were unable to resolve professional problems thoroughly.

The opportunity to apply for a center of excellence, which was given the name INFEKTZOON – the Center for excellence for animal infectious diseases and zoonoses (abbreviated INFEKTZOON) – and financed from EU structural funds made possible our effort to integrate research. The goal of the INFEKTZOON project is to solve contemporary problems involving bacterial, viral and parasitical animal diseases and zoonoses more thoroughly than has been thus far done through the joining of three departments – the Department of microbiology and immunology, the Department of epizootiology and parasitology, and the Department of pathological anatomy and pathological physiology. Aside from classical methodological approaches, we have shifted genomic and proteomic methods to the forefront. It is thus possible to resolve scientific problems complexly – from analysis of the epizootiological situation on farms and in the field through the isolation of microorganisms to the study of host-agent interactions with the use of the genomic and proteomic techniques, that is, by integration of classical approaches with the most modern methods of biological and medical research. Such an approach is effectively used at top laboratories abroad. Even in its germinal form INFEKTZOON creates the foundations for possible integration of our laboratories to the international research projects.

The project for our center of excellence was supported in part with 1,293,088.98 euro from European Union resources beginning in March 2009. The purchase of top-quality equipment and adapted laboratory spaces together with enthusiastic researchers of different age categories under the leadership of scientific guarantor Prof. Ing. Štefan Vilček, DrSc., put our research on a qualitative higher quality level. The scientific team can in its research rely on the full support of workers from the department of project cooperation and the department of information and communications technology under the leadership of project manager JUDr. Silvia Rolfová.

The mission and goals of INFEKTZOON

The mission of INFEKZOON is to be the leading scientific workplace in the East Slovakia region and in all of Slovakia in the battle with zoonoses and infectious diseases of animals and people according to the current needs of the epizootological-epidemiological situation in the state, the neighboring Central European regions and the EU. The top scientific level of the workplace ensures protection for agricultural and social animals from dangerous infectious diseases, leading to significant economic gains and improvements in the quality of animal welfare. The battle against animal infectious diseases also leads to better quality and a safer food-products supply chain for our citizens. In the case of zoonosis the workplace contributes to a significant degree to the public health of the human population. The training of highly qualified PhD students, veterinary doctors and diagnostic workers from practice raises the overall professional character of the Slovak veterinary community.

The INFEKTZOON scientific team is resolved to enforce its scientific research in the field of zoonosis and infectious and invasive diseases of animals to an international level, which will have the effect of creating optimal foundations for joining our scientific workers and teams to international projects. Doing research in a center of excellence on a level comparable with that of Europe helps prevent a brain-drain from Slovakia, because young science workers will be able to build their scientific careers in a domestic workplace.

The Institute of Parasitology of the Slovak Academy of Sciences in Košice – a partner in the center of excellence

In view of the fact that the scientific capacity of our university has its limits and in order that we thoroughly cover important aspects of the study of infectious diseases of animals and people, we invited workers from The Institute of Parasitology of the Slovak Academy of Sciences in Košice to the center of excellence.

The role of partner which has a preferential position in the field of research of parasitic zoonosis in Slovakia is to expand research on animal infectious diseases by important parasitozoonoses (echinococcus, trichinellosis, toxoplasmosis, and others) from two viewpoints. The first part of the research is focused on improving laboratory diagnostics of parasitic zoonosis not only by refining classic techniques like the ELISA tests and Western blotting, but also through the use of preparations of specific antigens by methods of the recombinant DNA technology for the newly developed ELISA tests. The second part of the research is focused on the study of host-parasite interactions on the immunological level and on the use of non-specific immunomodulation against helminthiasis in humans and animals.

Cooperation as the basis for high quality research

Cooperation of the individual sections of INFEKTZOON overcomes the isolation which before now was a disadvantage of our research and also opens a space for other work groups, primarily from clinical workplaces, genetics, biology, food hygiene, ecology and pharmaceutical research. Obviously, our partners for cooperation on projects are other universities and research institutes of the Slovak Academy of Sciences, non-university workplaces on the level of regions, the state as well as on the international level. Workers from the State Veterinary and Food Administration of the Slovak Republic and its institutes, their diagnostic laboratories as well as practicing veterinary doctors and other breeders are also in close cooperation.

INFEKTZOON as higher added value for research work

INFEKTZOON lays the foundations for the education of highly qualified PhD students and interdisciplinary specialist in pre- and post-graduate study, for the founding of informal scientific cooperation between work teams, the creation of new teams, the publishing of results in international scientific journals and higher quality solutions for practical problems in the area of infectious diseases of animals and zoonosis on the state level. Such innovative workplaces can be competitive for obtaining new grants on the international level, with the ambition of joining the EU's 7th and 8th framework programs.

Organization of the center of excellence and professional profiles of section heads

I. Section of pathogens isolation

The role of this section is the isolation and cultivation of viruses, bacteria and parasites from clinical material taken from diseased animals and their environment. Their preliminary identification is first performed using classic methods (e.g. cultivation, antigen and serological tests) and at the same time microorganisms from new vectors, which are carriers of new or newly emerging diseases are isolated here. Microorganisms so identified provide the first information about the diagnosis of infectious diseases and at the same time are material for additional, for example, genetic analysis of a pathogen and its protein products.

In the present period we are oriented on the isolation of viruses from animals suspected of having rabies, pestivirus infections of ruminants, herpes virus infections of small animals, porcine circovirus syndrome (agent PCV-2), porcine respiration and reproductive syndrome (agent PRRSV), *E. coli* infections and parasites causing zoonosis. Work in this laboratory will be operatively modified for current diseases of animals with special attention on zoonoses.

Head of section:
Prof. MVDr. Juraj Pistl, PhD.
Contact: mobil: +421 915 984 588
e-mail: pistl@uvlf.sk

Prof. MVDr. Juraj Pistl, PhD., is a scientific-pedagogical employee at the Department microbiology and immunology of University of Veterinary Medicine and Pharmacy in Košice. He is the head of the subjects microbiology, applied virology, clinical microbiology and immunology in a number of study programs. He is the guarantor of study program 4.2.13 virology for the 3rd degree of university education. His scientific activities are focused on the solving of current problems in the fields of microbiology and immunology, where he has shared in the planning of research tasks related to viruses (rotaviruses, pestiviruses, circoviruses) and bacterial infections (salmonelosis, actinobacillus) as well as problems of the environment (immunotoxicology). The result of these research activities is, in addition to dozens of scientific and professional publications and chapters in a scientific monographs and books, also the patent "A method for stabilization of leukocyte dialysate".

Prof. Pistl was the responsible leader for a number of domestic projects and is a member of the research teams of a number of important foreign projects in programs like the 6th RP of the EU, the Norwegian Financial Mechanism and Tempus. He has completed a number of foreign study programs (England, Italy, Belgium, Germany). He is a member of the Scientific Council of UVMPh in Košice and a member of the commission for defense of doctoral works and study programs 4.2.7 – Microbiology and 4.2.15 – Immunology.

Some significant scientific publications:

POLLÁKOVÁ, J., CSANK, T., PILIPČINCOVÁ, I., PISTL, J.: Comparative study of various cell lines susceptibility to cytopathic and non-cytopathic strains of Bovine viral diarrhea virus 1 and 2. *Acta Virol.*, 53, 4, 2009.

HOLOVSKÁ V., PISTL, J., KOVALKOVIČOVÁ N.: In vitro effect of pesticides (dichlofluanid, endosulfan, simazine, tolylfluanid and triallate) on proliferative activity of animal derived cell cultures. *Acta Biol. Hung.* 58, 61-74, 2007.

PISTL, J., KOVALKOVIČOVÁ, N., HOLOVSKÁ, V., LEGÁTH, J., MIKULA, I.: Determination of the immunotoxic potential of pesticides on functional activity of sheep leukocytes *in vitro*. *Toxicology*, 188, 73-81, 2003.

II. Section of molecular epizootiology and molecular diagnostics

The role of this section is the solving of current epizootological problems with modern methodological approaches. Aside from classical approaches we widely use molecular-genetic methods, primarily methods for sequencing of nucleic acids, computer analyses of molecular-genetic data and the construction of phylogenetic trees, which serve to identify the source of a contagion, the direction of the outbreak spreading and the more exact typing of microorganisms. The knowledge acquired leads to an improvement in understanding of the overall epizootiological situation and in the end results in the approval of more effective measures.

We focus the molecular diagnostic of infectious diseases and parasitosis on the development of new, more sensitive and more specific tests for detection of pathogens on the genetic level. Among the methodological approaches used are PCR, RT-PCR, real-time PCR, and their different modifications, quantitative real-time PCR, isothermal amplification of nucleic acids and other techniques.

At present we are concentrating our research on PMWS, PRRS, pestivirus infections of ruminants, herpes virus infections of dogs, salmonellosis and certain parasithosis.

MVDr. Anna Jacková, PhD., is a professional assistant of the Department of Epizootiology and Parasitology at University of Veterinary Medicine and Pharmacy in Košice. MVDr. Jacková worked for 15 years as an independent scientific worker with scientific-qualification degree IIa in the field of classical microbiology at the research institute of the Slovak Ministry of Agriculture (the Research Institute of Veterinary Medicine in Košice). Her current professional scientific work is devoted to the detection of viruses by using molecular-genetic methods and the development of new modern detection methods. She is oriented on the development of molecular epizootiology and molecular diagnostics of important viral diseases of animals.

She is the co-manager of a number of domestic (VEGA, APVV) and foreign scientific grants (Specific supporting action in the 6th EU framework program, the Switzerland-Slovakia-Ukrainian grant – SCOPES project). In addition to the organization of domestic scientific conferences, she was a member of the organizing committee for the ESVV workshop „Application of molecular-genetic methods in veterinary diagnostic virology“, which was organized in Košice.

Head of section: MVDr. Anna Jacková, PhD.
Contact: +421 915 984 648
e-mail: jackova@uvlf.sk

Some significant scientific publications:

VILČEK, Š., KOLESÁROVÁ, M., JACKOVÁ, A.: Genetic variability of bovine viral diarrhoea virus subtypes at 3´-nontranslated region. *Virus Genes*, 34, 31-35, 2007.

JACKOVÁ, A., NOVÁČKOVÁ, M., PELLETIER, C., AUDEVAL, C., GUENEAU, E., HAFFAR, A., PETIT, E., REHBY, L., VILČEK, Š.: The extended genetic diversity of BVDV-1: Typing of BVDV isolates from France. *Vet. Res. Commun.*, 32, 7-11, 2008.

VILČEK, Š., VLASÁKOVÁ, M., JACKOVÁ, A.: LUX real-time PCR assay for the detection of porcine circovirus type 2. J. *Virol. Methods* 165, 216-221, 2010.

III. Section of host-pathogen interactions

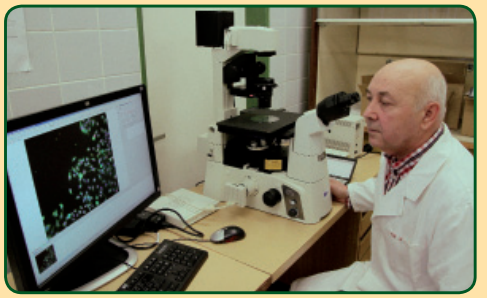
Research in this section is focused on the study of cell-pathogen and animal-infection interactions with the goal of recognising the immunological response of the host. At present, the strengthening of the immune response in animals against infectious pathogens by preventive addition of probiotics and natural immunostimulators is being studied in this section. Some factors of non-specific immunity, e.g. cytokines, are monitored via the expression of the relevant mRNA. In addition to the non-specific immunity, attention is also devoted to the evaluation of vaccines against a known zoonosis – rabies.

For the purposes of research, tests are used for the evaluation of selected immunological parameters, immunohistochemistry tests, flow-cell cytometry, classical PCR and quantitative real-time PCR. Gnotobiological animals are used not only on verifying the effects of biologically active substances but also on experimental infection of animals.

Prof. MVDr. Mikuláš Levkut, DrSc., is a scientific-pedagogical employee in the Department of Pathological Anatomy and Pathological Physiology at University of Veterinary Medicine and Pharmacy in Košice. His work is devoted to cell-bacteria interactions and mycotoxins. He studies quantitative and qualitative changes of immunocompetent cells in the intestinal system and in organs (spleen, blood). In the scope of these interactions he also researches quantitative changes in the products of the host cells being monitored (for example cytokines).

Prof. Levkut has been the responsible leader for a number of domestic and foreign grants, e.g. an EEC financed grant (1995) and an Italian-Slovak grant (1996-1999). He has completed long-term (Italy, Greece) and a number of short-term visits (Great Britain, Belgium, USA, Germany, Libya and others) at workplaces with a similar research orientation. He has published scientific papers in 99 CC journals. His works have been cited in more than 300 SCI journals.

He is the president of the UPJŠ Košice ethics committee, chairman of the state commission for defence of medical work in the field of food hygiene, infectious diseases and zoohygiene, a member of two state commissions for defence of medical work in the field of immunology, and other related branches of veterinary science. He is the chairman of the state commission for the defence of doctoral work in the field of immunology.



Head of section:
Prof. MVDr. Mikuláš Levkut, DrSc.
Contact: mobil:+421 915 984 705
e-mail: levkut@uvlf.sk

Some significant scientific publications:

HERICH, R., LEVKUTOVÁ, M., KOKINČÁKOVÁ, T., REITEROVÁ, K., HIPÍKOVÁ, V., LEVKUT, M.: Diagnosis and manifestation of encephalitozoonosis in mice after experimental infection with different species and application of dexamethasone. *J. Vet. Med. A,* 53, 340-345, 2006.

REVAJOVÁ, V., LEVKUT, M., ALDAWEK, A. M., HERICH, E., DVOROŽŇÁKOVÁ, E., KRUPICER, I.: Immunological changes after multiple Toxocara canis infection of lambs. *Helmithologia*, 43, 69-75, 2006.

REVAJOVÁ, V., PISTL, J., LEVKUT, M., MARCIN, A., LEVKUTOVÁ, M.: Influence of oregano and salvia extracts on lymphocyte subpopulation and functional activity of blood phagocytes and lymphocytes in chickens. *Food Agricult Immunol,* 21, 307-316, 2010.

IV. Section of genomics

The role of this section is the deeper study of the genomes of pathogens, primarily in strains which are responsible for atypical clinical symptoms or in newly discovered pathogens. We concentrate our attention on the analysis of genome organization, significant mutations with a response in the phenotype of a pathogen or clinical symptoms and the study of the molecular basis of virulent pathogens. In the near future the interaction of a pathogen with the cell will be studied on the level of the transcriptome.

From a methodological point of view we use methods of sequencing short and long fragments of DNA, PDFG and SSP. Our rich experience with the hybridization of nucleic acids and *in vitro* amplification of gene fragments logically leads to the introduction of microarray methods as one of the key methods of genomics. Here significant interconnections are also made with research in pharmacy, the teaching of which has already been established at our university. Data obtained from the microarray offers information which will be linked with proteomic research.

Head of section and scientific guarantor of the center of excellence:
Prof. Ing. Štefan Vilček, DrSc.
Contact: mobil: +421 815 984 654
e-mail: vilcek@uvlf.sk
personal Web page:
http://www.uvlf.sk/pages/stefan.vilcek/?q=authors/vilcek.html

Prof. Ing. Štefan Vilček, DrSc., is a scientific guarantor of the center of excellence, scientific employee at the Department of Epizootology and Parasitology at University of Veterinary Medicine and Pharmacy in Košice.

His scientific work is devoted to the development of new modern methods for the detection of genetic material (DNA, RNA) of viruses. He is focused on the analysis of animal viral diseases with the use of molecular-genetic methods and the development of molecular epizootiology of animal viral diseases. Another direction for his research is the analysis of viral genomes with the aim of obtaining new information on the character of viruses and data useful for sanitation programs against animal viral diseases.

Prof. Vilček has been the responsible leader of a number of domestic and foreign scientific grants (e.g. the Welcome Trust grant, a thematic network in the 5th EU framework program – head for Slovakia, Specific supporting action in the 6th EU framework program – head for Slovakia, a German-Slovak grant, a Switzerland-Slovakia-Ukrainian grant and others). He has completed long-term (Great Britain, Sweden, USA) and a number of short-term visits (Germany, France, Spain, Egypt, India and others) in scientific laboratories with similar science programs. He has published 90 scientific works listed in the PubMed database, and his works have been cited more than 1300 times in SCI journals; his Hirsch index H = 20, and he is the owner of 7 Czechoslovak patents.

He is a member of the Scientific Council of UVMPh in Košice, the Scientific Council of the Faculty of Natural Sciences at UPJŠ in Košice, a member of the UNESCO bioethics committee in Slovakia, a member of the state commission for defence of doctoral works in molecular biology, a member of the editorial board for the international scientific journal Virus Genes (Springer) and Veterinarni Medicina (Praque), a member of the editorial board of the scientific-popular journal Vesmír (Space) in Prague, was a member of the international committee of the European Society for Veterinary Virology (ESVV) and other professional associations.

Some significant scientific publications:

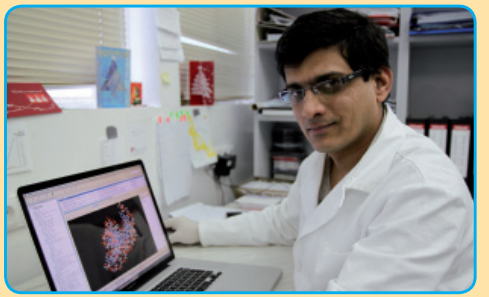
VILČEK, Š., ĎURKOVIČ, B., KOLESÁROVÁ, M., GREISER-WILKE, I., PATON, D.: Genetic diversity of international bovine viral diarrhoea virus (BVDV) isolates: identification of a new BVDV-1 genetic group. *Vet. Res.* 35, 609-615, 2004.

VILČEK, Š., RIDPATH, J. F., van CAMPEN, H., CAVENDER, J. L., WARG, J.: Characterization of a novel pestivirus originating from a pronghorn antelope. *Virus Res.,* 108, 187-193, 2005.

VILČEK, Š., WILLOUGHBY, K., NETTLETON, P., BECHER, P.: Complete genomic sequence of a border disease virus isolated from Pyrenean chamois. *Virus Res.,* 152, 164-168, 2010.

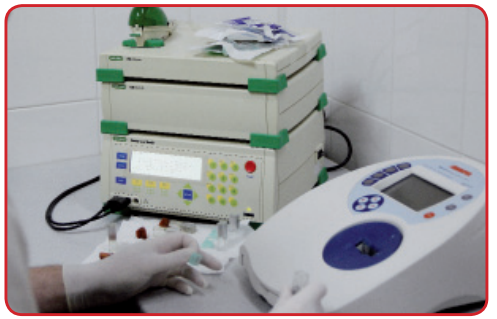
V. Section of proteomics

Proteomics was operationally classified as an independent section regarding complex research of pathogens and molecular mechanisms of infectious diseases. Research here is orientated on analysis, sequencing and identification of biologically significant protein pathogens and host cells which have significant relations to the development of the infection process. This research will continue the study of protein-protein interactions which are the roots to a deeper understanding of many biological activities of macromolecules. Research is at present primarily orientated on borreliosis. Among major methodological approaches, we mention the identification of proteins with two-dimensional electrophoresis and mass spectrometry (MALDI--TOF).



Head of section: MVDr. Mangesh Bhide, PhD.
Contact: mobil: +421 915 984 604
e-mail: bhide@uvlf.sk

MVDr. Mangesh Bhide, PhD., has worked for eight years in the field of Lyme borreliosis and tularemia. He specializes in advanced molecular techniques such as qRT-PCR, ligand binding assays, random mutagenesis, detection of mutations, DNA sequencing, far-western blotting, 2D electrophoresis, MALDI-TOF-TOF mass finger-printing, M2H assays, etc. In 2007 he completed his post-doctorate work (Marie-Curie post doctoral fellowship) in the field of host-pathogen (*Borrelia*) interactions related to factor H proteins 18 types of animals and 19 types of borreliosis. He has published 31 times in international journals (10 articles in the field of borreliosis and 3 articles in the field of francisellosis); a total of 120 citations, and 45 presentations at international conferences. He has worked on seven scientific projects as a co-worker, on three as a representative of the project leader and has led two projects (Marie-Curie International Incoming European fellowship) in the field of borreliosis, in National Institute of Health, Madrid, Spain. At present he is actively participating in two EU structural projects. MVDr. Bhide is also a co-manager of two *COST actions projects* (European science foundations).



Some significant scientific publications:

BHIDE, M. R., MUCHA, R., MIKULA Jr., I., KIŠOVÁ, L., SKRABANA, R., NOVÁK, M., MIKULA, I: Novel mutations in TLR genes cause hyporesponsiveness to Mycobacterium avium subsp. paratuberculosis infection. *BMC Genetics,* 10, 21, 2009.

BHIDE, M. R., CHAKURKAR, E., TKÁČIKOVÁ, L., BARBUDDHE, S., NOVÁK, M., MIKULA, I.: IS900-PCR-based detection and characterization of Mycobacterium avium subsp. paratuberculosis from buffy coat of cattle and sheep. *Vet. Microbiol.,* 112, 33-41, 2008.

BHIDE, M. R., TRÁVNIČEK, M., LEVKUTOVÁ, M., ČURLÍK, J., REVAJOVÁ, V., LEVKUT, M.: Sensitivity of Borrelia genospecies to serum complement from different animals and human: A host-pathogen relationship. *FEMS Immunol. Med. Microbiol.,* 43, 165-172, 2005.

MVDr. Emília Dvorožňáková, PhD., is the head Department of parasitozoonoses at The Institute of Parasitology of Slovak Academy of Sciences in Košice. In her scientific work she has dealt with the study of pathogenesis and diagnostics of parasitozoonoses, the role of immunocompetent cells and their cytokine production during immunomodulation and therapy of helminthiasis. She focuses on the cell-mediated immune response of the host toward a parasite, dependent on the interactions of the T-lymphocytes and macrophages.

She was the leader of the domestic APVV and VEGA projects, inter-academic cooperation with the Polish Academy of Sciences, and participated in the resolution of the international project of the 5th framework program of the EU (TRICHIPORSE) and bilateral projects with the Italian University La Sapiensa in Rome. She took part in a study stay in Japan supported by the Japan Society for Promotion of Science at the Tokyo Medical and Dental University, completed a stage for the Institute of Microbiology and Parasitology of the Czech Academy of Sciences in Czech Republic and at the workplace of the Netherlands Vaccine Institute in Bilthoven, The Netherlands. She has published 33 scientific papers and has been cited 97 times in the SCI database. She is a member of the Scientific Council of the Institute of Parasitology of Slovak Academy of Sciences in Košice and a member of the editorial board of the international scientific journal Helminthologia (Springer-Versita).

Some significant scientific publications:

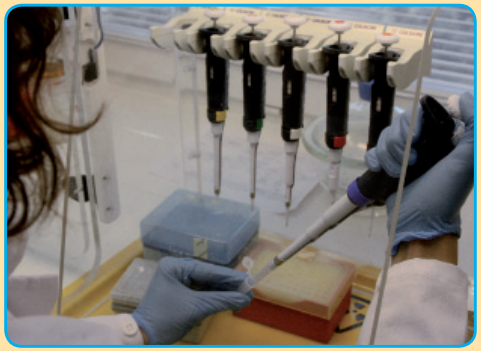
DVOROŽŇÁKOVÁ, E., PORUBCOVÁ, J., ŠEVČÍKOVÁ, Z.: Immune response of mice with alveolar echinococcosis to therapy with transfer factor, alone and in combination with albendazole. *Paras. Res.,* 105, 1067-1076, 2009.

KOLODZIEJ-SOBOCINSKA, M., DVOROŽŇÁKOVÁ, E., DZIEMIAN, E.: *Trichinella spiralis*: Macrophage activity and antibody response in chronic murine infection. *Exp. Paras.,* 112, 52-62, 2006.

DVOROŽNÁKOVÁ, E., HRČKOVÁ, G., BOROŠKOVÁ, Z., VELEBNÝ, S., DUBINSKÝ, P.: Effect of treatment with free and liposomized albendazole on selected immunological parameters and cyst growth in mice infected with *Echinococcus multilocularis*. *Parasitology Int.,* 53, 315-325, 2004.

Partner representative – The Insitute of Parasitology of Slovak Academy of Sciences in Košice: MVDr. Emília Dvorožňáková, PhD.

Contact: +421 55 633 14 11
e-mail: dvoroz@saske.sk



Abstracts of the more important scientific works of INFEKTZOON staff

(The underlined authors are scientists with INFEKTZOON)

POLLÁKOVÁ, J., CSANK, T., PILIPČINCOVÁ, I., PISTL, J.: Comparative study of various cell lines susceptibility to cytopathic and non-cytopathic strains of Bovine viral diarrhea virus 1 and 2. *Acta Virol.*, 53, 287-289, 2009.

Bovine viral diarrhea virus 1 and 2 (BVDV 1 and 2) (the genus *Pestivirus*, the family *Flaviviridae*) is distributed worldwide and causes severe economical losses due to the decreased fertility, abortions, diarrhea, respiratory symptoms, and persistent infection in intrauterinary infected calves. The aim of this study was to compare the susceptibility of different cell lines derived from various tissues to non-cp and cp strains of BVDV. We examined five cell lines: Bovine embryonic lungs (BEL), bovine turbinated (BT), Madin-Darby bovine kidney (MDBK), calf oesopharyngeal (KOP-R), and sheep fetal thymus (SFT-R), which were inoculated with two cp BVDV strains (NADL and Oregon C24V) and two non-cp BVDV strains (PT810 and CS8644). According to titer of virus, the KOP-R cell line was the most susceptible for the isolation of cp BVDV strains and SFT-R and BEL cell lines for non-cp BVDV strains. Any of the cell cultures tested was universal for all BVDV strains. Paradoxically, the lowest harvest of all BVDV strains tested was found in the cell line MDBK, which is one of the most frequently used cell line for the isolation of BVDV.

PISTL, J., NOVÁČKOVÁ, M., JACKOVÁ, A., POLLÁKOVÁ, J., LEVKUT, M., VILČEK, Š.: First evidence of porcine circovirus 2 (PCV2) in Slovakia, *Deutsche Tierärztliche Wochenschrift*, 116, 19-23, 2009.

Circoviruses belong to the family of small DNA viruses, which infect a number of animal species. The most important circovirus in swine is porcine circovirus type 2 (PCV2). The most frequent clinical form of circovirus infection in swine is the post-weaning multisystemic wasting syndrome (PMWS). Because Slovakia is one of the countries with unknown prevalence of PMWS, we focused on the detection of Slovak PCV2 isolates from pigs with PMWS-like symptoms (clinical symptoms, pathology) based on generally accepted scientific criteria (biological and molecular-genetic methods). The affected weaned pigs showed general symptoms characteristic for PMWS with pathological-anatomical changes particularly in lungs, lymph nodes and kidneys. Immunohistochemistry and PCR allowed us to prove PCV2 in cryosections of organs from infected animals. Immunoperoxidase test (IPMA) was used to confirm PCV2 in cells of cell culture PK-15 with the highest titre of the virus found in the lymph node tissue. Sequencing proved the specifity of PCR products. BLAST analysis indicated that nucleotide sequences of PCV2 amplified fragments were the most similar to the Austrian isolate of PCV2. This paper presents the first detection and isolation of PCV2 in Slovakia, which was confirmed by laboratory methods at antigenic and genetic level.

VILČEK, Š., VLASÁKOVÁ, M., JACKOVÁ, A.: LUX real-time PCR assay for the detection of porcine circovirus type 2. *J. Virol. Methods* 165, 216-221, 2010.

Light Upon eXtension real time PCR (LUX real time PCR) assay was developed for the detection of porcine circovirus type 2 (PCV2). The primers flanking a 114 bp fragment were selected from ORF1. The optimized assay could detect 20 viral copies of pBluescript SK+ plasmid containing inserted PCV2 DNA. The dynamic range of quantitative analysis covered a 7-order interval ranging from 20 to 2.10^8 genome equivalents per assay with the best result in the range from 2.10^2 to 2.10^7 viral copies. The LUX real time PCR assay had a high specificity since it detected PCV2 but not PCV1, CSFV, PRRSV or negative samples. There was good agreement between LUX real time PCR and conventional PCR when lymph nodes from PCV2 infected animals were tested. A comparison of LUX real time PCR with TaqMan PCR and SYBR Green PCR indicated that the amount of viral copies determined using linear calibration curve differed from assay to assay but not more than an order. LUX real time PCR, similar as TaqMan PCR, was more specific in generation of fluorogenic signal than SYBR Green PCR.

GOLDOVÁ, M., TÓTH, Š., LETKOVÁ, V., MOJŽIŠOVÁ, J., KOŽAROVÁ, I., POMFY, M.: Comparison of the histological methods in the diagnostic of deer cysticercosis. *Helmintologia*, 45, 121 – 125, 2008.

Histochemical methods for the detection and diagnosis of the developmental stages of the canine tapeworm, from the genus *Taenia* found in the heart and lungs of red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) hunted in Eastern Slovakia, is presented here. Detailed morphology of cysticerci (*Cysticercus* spp.), based on microscopic and histochemical

analysis is described. For confirmation and demonstration of PAS-positive substances in the body of parasitic tissue (tegument and mesenchyme) the McManus - PAS method was used. The histochemical method according to Van Kossa was very effective for confirmation of calcareous corpuscles, which are one of the most important histological markers of cestode tissues (larva or adult).

JACKOVÁ, A., NOVÁČKOVÁ, M., PELLETIER, C., AUDEVAL, C., GUENEAU, E., HAFFAR, A., PETIT, E., REHBY, L., VILČEK, Š.: The extended genetic diversity of BVDV-1: Typing of BVDV isolates from France. *Vet. Res. Commun.*, 32, 7-11, 2008.

Of 47 BVDV-1 isolates grouped in the 5´-UTR phylogenetic tree, 9 isolates fall into BVDV-1b, 2 isolates into BVDV-1d and 33 isolates into the BVDV-1e group. Three isolates formed a new phylogenetic group indicating new, the twelfth BVDV subtype "l" - BVDV-1l. The typing of viruses in 5´-UTR was also confirmed in Npro region with 14 selected isolates, including all 3 isolates grouped in BVDV-1l cluster. Our study demonstrates that the analysis of broader collection of BVDV isolates may result in the higher genetic diversity of BVDV-1 and new viral subtypes could be identified. The study of genetic diversity of BVDV isolates is useful for the understanding of pestivirus evolution as well as for molecular epidemiology.

VLASÁKOVÁ, M., JACKOVÁ, A., VILČEK, Š.: Genetic typing of porcine circovirus type 1 (PCV-2) isolates from Slovakia. *Res. Vet. Sci.*, 90, 168-173, 2011.

Of 120 clinical specimens obtained from pigs breed on 28 PMWS-affected farms in Slovakia, PCV-2 DNA was detected by single PCR in 77 samples. A short 224 bp fragment of ORF2 was used for preliminary grouping of isolates by phylogenetic analysis. Nucleotide sequences of the entire ORF2 region provided more precise genetic typing and segregation of preselected isolates (n=10) into two known PCV-2a (n=1) and PCV-2b (n=9) genotypes. Recognising of the complete genome sequences of three selected isolates was useful for their definitive grouping into genotypes PCV-2b, cluster 1A and PCV2a, cluster 2D. No correlation between the mutations and geographic origin of isolates was observed.

LEVKUT, M., REVAJOVÁ, V., LEVKUTOVÁ, M., ŠEVČÍKOVÁ, Z., HERICH, R., BORUTOVÁ, R., LENG, L.: Leukocytic responses of broilers following dietary contamination with deoxynivalenol and/or treatment by dietary selenium supplementation. *Brit. Poult. Sci.*, 50, 181-187, 2009.

1. This experiment was to investigate the effects of natural dietary contamination with a mycotoxin product (deoxynivalenol: DON) and/or with dietary selenised yeast (Se-yeast), on respiratory burst and phagocytic activity of granulocytes and the frequency of B- and T-lymphocytes in peripheral blood of broilers.
2. Sixty one-day-old chicks of both sexes were divided into 4 groups, each of 15 birds, fed on a control diet that contained 0.2mgDON/kg and 0.4mg Se/kg (CON group), a diet supplemented with 1mg Se-yeast/kg (Se--yeast group), a diet contaminated with 3mgDON/kg (DON group) or a diet contaminated with DON and supplemented with Se-yeast (DON plus Se-yeast group).
3. Blood samples collected from the birds at the age of 4 weeks showed that neither B- and T-cell numbers nor granulocytic respiratory burst were influenced by 3mg-DON/kg. Blood granulocyte phagocytic activity was not reduced by DON but numbers of heterophils were

increased. In the DON plus Se yeast group phagocytic activity was the same as in the CON group. The Se-yeast and DON plus Se-yeast groups had increased numbers of $CD3^+$, $CD4^+$, and $CD8^+$ T-cells as well as IgM^+ B-cells in their blood compared to both CON and DON--groups.
4. The results show there is no significant effect of dietary DON up to 3mg/kg on leukocytes apart from the compromised blood granulocytes phagocytic activity and increased numbers of heterophils. The increased numbers of B- and T-lymphocytes in blood of birds fed on diets with supplementation of organic Se indicates some positive effects of this essential microelement on poultry lymphoid cells.

KRÁĽOVÁ-HROMADOVÁ, I., ŠTEFKA, J., ŠPAKULOVÁ, M., OROSOVÁ, M., BOMBAROVÁ, M., HANZELOVÁ, V., BAZSALOVICSOVÁ, E., SCHOLZ, T.: Intra-individual internal transcribed spacer 1 (ITS1) and ITS2 ribosomal sequence variation linked with multiple rDNA loci: A case of triploid Atractolytocestus huronensis, the monozoic cestode of common carp. *Int. J. Paras.*, 40, 175-181, 2010.

Complete sequences of the ribosomal internal transcribed spacers (ITS1 and ITS2) and karyological characters of the monozoic (unsegmented) tapeworm Atractolytocestus huronensis Anthony, 1958 (Cestoda: Caryophyllidea) from Slovakia were analysed, revealing considerable intra-genomic variability and triploidy in all analysed specimens. Analysis of 20 sequences of each ITS1 and ITS2 spacer yielded eight and 10 different sequence types, respectively, In individual tapeworms, two to four ITS1 and three to four ITS2 sequence types were found. Divergent intra-genomic ITS copies were mostly induced by nucleotide substitutions and different numbers of short repetitive motifs within the sequence. in addition, triploidy was found to be a common feature of A. huronensis. The karyotype of Slovakian A. huronensis possesses three sets of chromosomes (3n = 24, n = 4m + 3st + 1 minute chromosome), similar to the previously described triploicly in conspecific tapeworms from North America. Fluorescent in situ hybridisation (FISH) with a ssrDNA probe revealed two distinct rDNA clusters for each homologue of the triplet number 2. To date, A. huronensis is the only cestode species in which intra-individual ITS sequence variants were found in parallel with its triploid nature and multiple rDNA loci. Some of these molecular and genetic features were observed in several other species of basal or nearly basal tapeworms of the orders Caryophyllidea and Diphyllobothriidea, which indicates that the phenomena may be characteristic for evolutionarily lower tapeworms and deserve more attention in future studies.

HRČKOVÁ, G., VELEBNÝ, S., SOLÁR, P.: Dynamics of hepatic stellate cells, collagen types I and III synthesis and gene expression of selected cytokines during hepatic fibrogenesis following Mesocestoides vogae (Cestoda) infection in mice. *Int. J. Paras.*, 40, 163-174, 2010.

In the present Study, the relationship between progression of Mesocestoides vogae infection in the liver of mice, the accumulation rate of collagen types I and III, gene expression of fibrogenic factors and cytokines was examined within 6 weeks p.i. Due to asexual multiplication, the total number of larvae in the liver increased considerably and 63.4 % were found in collagen Capsules on day 42 p.i. Intense staining for both collagens was recorded in the activated hepatic stellate cells (HSCs) throughout the period of this study in the inflammatory lesions. With progressing infection, cellular expression of both collagens was confined to the flat cells, myofibroblasts, which were scattered among collagen fibres in parenchymal lesions and capsules. Collagen-positive areas mirrored immunostaining of alpha-smooth muscle actin (alpha-SMA) in HSCs and myofibroblasts. Gene expression of both collagens increased rapidly within 14 days p.i. and their expression pattern resembled that for pro-fibrotic cytokine transforming growth factor (TGF)--beta 1 and alpha-SMA protein. IL-10 cytokine expression was up-regulated following day 14 p.i. and that of IL-13 was up-regulated early p.i., then transcription elevated gradually mirroring the activity of other pro--fibrotic markers. In contrast, transcription activity of TNF-alpha and IFN-gamma was elevated shortly after infection, followed by the partial down-regulation of gene expression, indicating the lack of larval killing, enhanced granulomatous inflammation and the perpetuation of hepatic fibrosis. Histomorphometric analysis of the parenchymal fibrous lesions, surface areas of larvae surrounded with the inflammatory infiltrates and surface areas of developing or mature larva-containing granulomas, correlated with the proportion of free and encapsulated larvae, immunostaining and gene expression patterns of collagens and profibrotic markers. At a later stage of infection (day 28 p.i. onwards) collagen I-positive areas occupied a greater surface area and formed mature larval capsules and scars in the liver. In contrast, collagen III was less abundant and was localised mainly in the fibrous lesions in damaged parenchyma, Suggesting their specific up-regulation as the part of host-protecting and tissue-healing responses.

BRNA, M., REVAJOVÁ, V., ŽITŇAN, R., LEVKUT Jr., M., BARAN, M., LEVKUT, M.: Changes in integrin-positive cells and T cell subpopulations in the peripheral blood and intestine of calves fed soya protein. *J. Anim. Feed Sci.*, 19, 358-367, 2010.

In order to examine the relation of known intestinal lesions to changes in T-cell phenotypes and integrin expression, 16 male 10-day-old Holstein calves were divided into two groups. For 28 days of the experiment, eight males were fed NutriMilk in which 50 % of the crude protein was soya protein, and eight control animals, with NutriMilk containing only milk casein. The animals fed soya protein showed shorter jejunal villi with a corrugated surface and deeper crypts compared with the control calves. A higher density of CD8+ cells in the intestinal mucosa and a decrease of these cells in peripheral blood were found in calves fed soya protein. The number of CD11b-positive cells was decreased in the peripheral blood of calves fed soya protein. Lower expression of integrin could be related to the appearance of non-mature polymorphonuclear cells. It is not clear if the decrease in CD11b expression on blood cells could also be influenced by milk replacer, i.e. soya protein.

OBERHAUSEROVÁ, K., BAZSALOVICSOVÁ, E., KRÁĽOVÁ - HROMADOVÁ, I., MAJOR, P., REBLANOVÁ, M.: Molecular discrimination of eggs of cervid trematodes using the Teflon (PTFE) technique for eggshell disruption. *Helminthologia*, 47, 147-151, 2010.

Molecular comparative analysis of eggs of four liver and stomach flukes of cervids and domestic ruminants,

Fasciola hepatica, Fascioloides magna, Dicrocoelium dendriticum and Paramphistomum cervi, was performed using a new methodological approach for eggshell disintegration. Eggs of all species were crushed mechanically by the Teflon method (PTFE) without use of chemical reagents and an efficient disruption of eggshell was checked microscopically. The egg suspension was then subjected to DNA isolation and PCR amplification using species-specific primers that annealed to the internal transcribed spacer 2 (ITS2) region of ribosomal DNA. The size of PCR products of individual species corresponded well to the size of amplicons obtained from adult flukes. The results provided evidence that the Teflon method does not destroy the structure of egg DNA, thus making the procedure broadly applicable during coprological examinations. Molecular markers introduced here are particularly important for blanket screening and differentiation of morphologically hardly distinguishable *F. hepatica*, *F. magna* and *P. cervi* eggs.

HREŠKO, S., MOJŽIŠ, M., TKÁČIKOVÁ, Ľ.: Prion protein gene polymorphism in healthy and BSE-affected Slovak cattle. *J. Appl. Genet.* 50, 371–374, 2009.

Variation of the *PrP* gene was examined in healthy and BSE-affected Slovak cattle. According to previous studies, the 23-bp indel polymorphism is supposed to be associated with higher susceptibility to BSE. We investigated 301 samples from healthy cattle of various Slovak breeds and 24 samples obtained from tissues of BSE-affected cattle in Slovakia. We examined the *PrP* gene for the 23-bp indel polymorphism in the putative promoter region, 12-bp indel polymorphism in the first intron of the *PrP* gene, variations in number of octapeptide repeat units, and presence of the silent AAC›AAT transition in codon 192 within the protein-coding region of the *PrP* gene. Altogether we found 23 different genotypes in the group of healthy cattle and only 6 genotypes in the group of BSE-affected cattle. Comparison of homozygotes for the 23-bp insertion and heterozygotes showed significant differences ($P < 0.05$) in genotype distribution between the examined groups. Thereby the homozygous insertion genotype at the 23-bp indel polymorphism site in the promoter region of the prion protein gene seems to have a protective effect against BSE.

VILČEK, Š., WILLOUGHBY, K., NETTLETON, P., BECHER, P.: Complete genomic sequence of a border disease virus isolated from Pyrenean chamois. *Virus Res.*, 152, 164-168, 2010.

Pestivirus infections occur in cattle, sheep, pigs and numerous other species within the *Artiodactyla*. Here we report analysis of the full-length genome sequence of the pestivirus strain H2121 which was recently isolated from Pyrenean chamois and typed as border disease virus (BDV) genotype 4. Comparison with full-length genomic sequences of the approved pestivirus species Bovine viral diarrhea virus-1 (BVDV-1), BVDV-2, BDV, and Classical swine fever virus, as well as pestivirus strains Giraffe-1 and Th_04 confirmed that the chamois pestivirus strain is most similar to BDV. The viral genome of H2121 is 12,305 nucleotides long and contains one large open reading frame. The latter encodes a polyprotein consisting of 3,899 amino acids and is flanked with 376 nucleotides long 5′ untranslated region (UTR) and 229 nt long 3′ UTR. The genome organization of the chamois virus is reminiscent to that of other pestiviruses. Compared to other BDV strains including BDV-1 strain X818 and BDV-2 strain Reindeer-1, the 5′ UTR and ORF of the chamois virus are very similar in length, while the 3′ UTR of H2121 is 31 to 44 nucleotides shorter. In contrast to other BDV strains, the genome of the chamois virus contains a unique four amino acid insertion at the N-terminus of NS2. Apart from these differences, no further insertion/deletion or genetic rearrangement were present in the genome of the chamois pestivirus.

MIKULA, I. Jr., BHIDE, M. R., PASTOREKOVÁ, S., MIKULA, I.: Characterization of ovine TLR7 and TLR8 protein coding regions, detection of mutations and Maedi Visna virus infection. *Vet. Immunol. Immunopathol.*, 138, 51-59, 2010.

Toll-like receptors (TLRs) 2, 3, 4, 7, 8 and 9 play a crucial role in the recognition of viral entities and modulation of the innate immune system. This work presents sequence analysis of ovine TLR7 and TLR8 genes, depicts novel mutations and describes frequencies of mutations in Maedi Visna infected and healthy sheep. Totally 48 samples of the breed Tsigai were analyzed for the presence of mutations. Within 20 mutations, 14 were silent whereas 6 were missense. The frequencies of missense mutations in the Maedi Visna infected compared to non-infected sheep were: Lys115Glu (P - 0.766, F-test), Asn117 (P - 0.380) and Lys818Arg (P - 0.739). These three mutations were localized in extra LRR (lucine rich repeat) region of TLR7, while mutation Ile73Leu (P - 0.498) was located within LRR2 motif. Both mutations in TLR8, Asn165Lys (P - 1.0) and Tyr349His (P - 0.700), were present in extra LRR region. The secondary structure analysis of ovine TLR7 and TLR8 revealed conserved LRR motif structure, however with some irregularities compared to cattle and human. Transmembrane domains of TLR7 and TLR8 showed 100 % homology between sheep and cattle wherein no mutations were found. In both TLRs TIR domains were highly conserved with occurrence of 4 silent mutations. Mutations in TLR7 and TLR8 may play an important role as predisposition factor for Maedi Visna infection. Considering the sequence homology among sheep, cattle and human genes encoding TLR7 and TLR8, we predict their similar function, localization and downstream signaling.

MUCHA, R., BHIDE, M. R., CHAKURKAR, E. B., NOVÁK, M., MIKULA, I.: Toll-like receptors TLR1, TLR2 and TLR4 gene mutations and natural resistance to Mycobacterium avium subsp. paratuberculosis infection in cattle. *Vet. Immunol. Immunopathol.*, 128, 381-388, 2009.

Toll like receptors (TLRs) are a class of pattern recognition receptors belonging to the innate immune system. Mutations in the protein coding region of TLRs are associated with altered responsiveness to pathogen-associated molecular patterns (PAMPs). A search was performed for novel mutations in bovine TLR1, TLR2 and TLR4 genes associated with the Mycobacterium avium subsp. paratuberculosis (MAP) infection. The work was also focused on the assessment of linkage between well known mutations in TLR genes (TLR2: Arg677Trp, Pro681His and Arg753Gln; TLR4: Asp299Gly and Thr399Ile), and the susceptibility of cattle to MAP infection. Detection of MAP infection in cattle population (n = 711) was based on IS900 PCR, which

revealed 22.50 % (n = 160) MAP positivity. Known mutations in TLR2 and TLR4 genes were not found in cattle population. A novel mutation Val220Met was associated (Odd's ratio, OR-3.459) with increased susceptibility to MAP infection. Toll/interleukin-1 receptor (TIR) domain of TLR2 was screened for the presence of mutations, wherein a novel Ile680Val mutation was linked with MAP infection. In silico analysis of the bovine TLR4 ectodomain (ECD) revealed the polymorphic nature of the central ECD and irregularities in the central LRR motifs. LRR11 of the TLR4 showed five missense mutations possibly linked with the increased susceptibility to MAP infection. The most critical position that may alter the pathogen recognition of TLR molecule was 4th residue downstream to LRR domain. Two such missense mutations in TLR4 (Asp299Asn downstream to LRR11, and Gly389Ser downstream to LRR15) were associated with MAP infection. Briefly, the work describes novel mutations in the bovine TLRs and presents their association with the MAP infection.

BHIDE, M. R., MUCHA, R., MIKULA Jr., I., KIŠOVÁ, L., ŠKRABANA, R., NOVÁK, M., MIKULA, I.: Novel mutations in TLR genes cause hyporesponsiveness to Mycobacterium avium subsp. paratuberculosis infection. *BMC Genetics*, 10, art. no. 21, 2009.

Background: Toll like receptors (TLR) play the central role in the recognition of pathogen associated molecular patterns (PAMPs). Mutations in the TLR1, TLR2 and TLR4 genes may change the ability to recognize PAMPs and cause altered responsiveness to the bacterial pathogens. Results: The study presents association between TLR gene mutations and increased susceptibility to Mycobacterium avium subsp. paratuberculosis (MAP) infection. Novel mutations in TLR genes (TLR1-Ser150Gly and Val220Met; TLR2 - Phe670Leu) were statistically correlated with the hindrance in recognition of MAP legends. This correlation was confirmed subsequently by measuring the expression levels of cytokines (IL-4, IL-8, IL-10, IL-12 and IFN-γ) in the mutant and wild type moDCs (mocyte derived dendritic cells) after challenge with MAP cell lysate or LPS. Further in silico analysis of the TLR1 and TLR4 ectodomains (ECD) revealed the polymorphic nature of the central ECD and irregularities in the central LRR (leucine rich repeat) motifs. Conclusion: The most critical positions that may alter the pathogen recognition ability of TLR were: The 9th amino acid position in LRR motif (TLR1-LRR10) and 4th residue downstream to LRR domain (exta-LRR region of TLR4). The study describes novel mutations in the TLRs and presents their association with the MAP infection.

PULZOVÁ, L., BHIDE, M. R., ANDREJ, K.: Pathogen translocation across the blood-brain barrier. *FEMS Immunol. Med. Microbiol.*, 57, 203-213, 2009.

Neurological manifestations caused by neuroinvading pathogens are typically attributed to penetration of the blood-brain barrier (BBB) and invasion of the central nervous system. However, the mechanisms used by many pathogens (such as Borrelia) to traverse the BBB are still unclear. Recent studies revealed that microbial translocation across the BBB must involve a repertoire of microbial-host interactions (receptor-ligand interactions). However, the array of interacting molecules responsible for the borrelial translocation is not yet clearly known. Pathogens bind several host molecules (plasminogen, glycosaminoglycans, factor H, etc.) that might mediate endothelial interactions in vivo. This review summarizes our current understanding of the pathogenic mechanisms involved in the translocation of the BBB by neuroinvasive pathogens.

Unique instruments

Aside from specialized microscopes, automatic blood-cell counters, laminar boxes, equipment for processing histological samples, PCR thermocyclers, real-time PCR thermocyclers and others, the center of excellence has the following unique instruments:

Gnotobiotic isolator for rodents
Unique equipment designated for in vivo experiments involving gnotbiotic animals such as mice and rats. This facility has available all requirements which are essential for working with germ-free animals, like handling, feeding, cleaning, air-filtration as well as the disinfection and sterilization process.

Flow cytometer
The device is used for the analysis of cellular sub--populations.

Automatic electrophoresis system
A device for electrophoresis of nucleic acids and proteins on chips used to determine the quality of DNA, RNA and proteins in very small amounts.

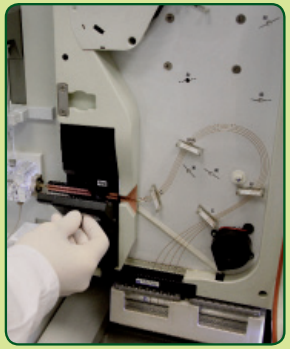
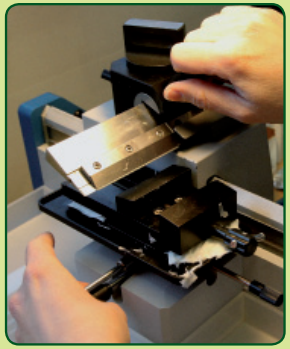
DNA sequencer
The device is used for the sequencing of DNA.

Hybridization station
Equipment used for hybridization of microarray.

Microarray scanner
A device used for scanning a microarray after hybridization experiments. Suitable for the study of differential expression of genes or differential diagnostics of infectious pathogens.

System for 2D electrophoresis
The device is used for the separation of proteins using two-dimensional chromatography.

MALDI-TOF
A device used for chromatographic and mass separation of proteins as well as the analysis of the mass spectra of peptides.


International projects:

Project SK0021 Norwegian Financial Mechanism: Biotechnological and natural products for the health of swine – quality and safe foodstuffs.
Project leader: Radomíra Nemcová, time period: 2007-2011.

COST Action FA1002: Farm Animal Proteomics, MoU: 4135/10.
Project leader: Mangesh Bhide (Slovakia), time period: 2010-2014

SCOPUS project No. IZ73Z0_128050/1: Epidemiology of bovine viral diarrhea in Ukraine: Development of a control program.
Project leader: Ernst Peterhans (Switzerland), Štefan Vilček (Slovakia), Anton Gerylovich (Ukraine), time period: 2010-2012.

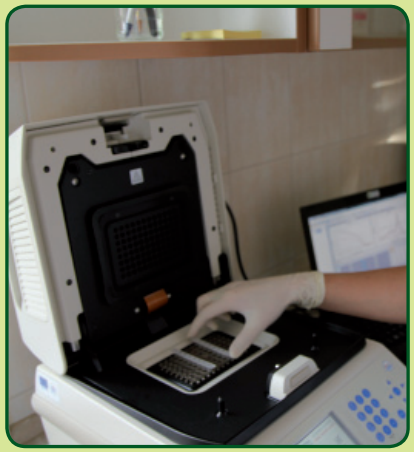
Offer for cooperation and services

INFEKTZOON offers potential partners cooperation in a number of areas:

Infectious and non-infectious experiments on gnotobiotic animals (mice, rats).
There is a long tradition of work with gnotobiotic animals in the Laboratory of gnotobiology at the Department of microbiology and immunology of UVMPh in Košice. Experiments on germ-free animals allows for the more detailed characterization of the organism-pathogen relation from both the microbiological and the immunological viewpoint. Such experiments have a special significance with evaluations of probiotics and substances with immunomodulation effects as factors used in the prevention of infectious diseases.
Contact: Prof. MVDr. Juraj Pistl, PhD. (pistl@uvlf.sk), MVDr. Radomíra Nemcová, PhD. (nemcova@uvlf.sk), MVDr. Soňa Gancarčíková, PhD. (gancarcikova@uvlf.sk).

Development of PCR and real-time PCR for the detection of microorganisms
Our group has great experience with the development of PCR, RT-PCR and nested-PCR for the detection of viruses. We have also experience with the development of real-time PCR assay diagnostics on the basis of SYBR-Green, TaqMan, but also with less common systems like LUX PCR and Plexor--PCR.
Contact: Prof. Ing. Štefan Vilček, DrSc. (vilcek@uvlf.sk), MVDr. Anna Jacková, PhD. (jackova@uvlf.sk), MVDr. Michaela Vlasáková (novackova@uvlf.sk)

Analysis of immunological profile of animals and analysis of cell sub-population
Our colleagues have good experience with the analysis of immunological profile of animals and have available a rich spectrum of method for its determination and for analysis of sub-populations of immunologically important cells.
Contact: Prof. MVDr. Jana Mojžišová, PhD. (mojzisova@uvlf.sk), Prof. MVDr. Mikuláš Levkut, DrSc. (levkut@uvlf.sk)



Molecular epizootiology of viral ainfections
We have good experience with the molecular epizootiology of viral diseases using nucleotide sequencing and phylogenetic analysis of molecular data.
Contact: Prof. Ing. Štefan Vilček, DrSc. (vilcek@uvlf.sk), MVDr. Anna Jacková, PhD. (jackova@uvlf.sk)

Sequencing of DNA
We offer services for the sequencing of DNA fragments using a DNA sequencer.
Contact: MVDr. Mangesh Bhide, PhD. (bhide@uvlf.sk)

Microarray technology
Our laboratory has complete microarray technology for the study of differential expressions of genes and the detection of microorganisms.
Contact: Prof. Ing. Štefan Vilček, DrSc. (vilcek@uvlf.sk)

Separation and analysis of proteins for proteomic research
We have instruments available as well as experience with proteomic research.
Contact: MVDr. Mangesh Bhide, PhD. (bhide@uvlf.sk)

The text was updated in February 2011.
Editors: Prof. Ing. Štefan Vilček, DrSc., Mgr. Ľudmila Kundríková. Contributors: Prof. MVDr. Juraj Pistl, PhD., Prof. MVDr. Mikuláš Levkut, DrSc., MVDr. Anna Jacková, PhD., MVDr. Mangesh Bhide, PhD., MVDr. Emília Dvorožňáková, PhD. Photo: Prof. Ing. Štefan Vilček, DrSc. Graphics: Agentúra YOURS. Printed by: Rotaprint s.r.o.