FOLIA VETERINARIA

The scientific journal of the UNIVERSITY
OF VETERINARY MEDICINE IN KOŠICE
— The Slovak Republic

ISSN 0015-5748

supplementum
XLVIII • 2004

UNIVERSITY
OF VETERINARY MEDICINE
IN KOŠICE

EDITORIAL BOARD

FOLIA VETERINARIA is issued by the *University of Veterinary Medicine* in Košice (UVL); address: Komenského 73, 041 81 Košice, The Slovak Republic (tel.: +421 55 633 51 03, fax: +421 55 633 51 03, E-mail: Simkova@uvm.sk).

The journal is published quarterly in English (numbers 1—4) and distributed worldwide.

Subscription rate for 1 year is 200 Sk, for foreigners 80 euros. Orders are accepted by *The Department of The Scientific Information — The Library of The University of Veterinary Medicine, Košice* (UVIK); the subscription is accepted by the National bank of Slovakia in Košice (at the account number mentioned below).

Bank contact: *National bank of Slovakia*, 040 01 Košice, Strojárenská 1, our account number: 19-1924-512/0720.

FOLIA VETERINARIA, vydáva *Univerzita veterinárskeho lekárstva* v Košiciach (UVL), Komenského 73, 041 81 Košice, Slovenská republika (tel.: 055/633 51 03, fax: 055/633 51 03, E-mail: Simkova@uvm.sk).

Časopis vychádza kvartálne (č. 1—4) a je distribuovaný celosvetove.

Ročné predplatné 200 Sk, pre zahraničných odberateľov 80 eur. Objednávky prijíma *Ústav vedeckých informácií a knižnice Univerzity veterinárskeho lekárstva v Košiciach* (UVIK); predplatné Národná banka Slovenska v Košiciach (na nižšie uvedené číslo účtu).

Bankové spojenie: Národná banka Slovenska, Košice, Strojárenská 1, číslo príjmového účtu: 19-1924-512/0720.

Tlač: **EMILENA**, Čermeľská 3, 040 01 Košice
Sadzba: **Aprilla,** s.r.o., Hlavná 40, 040 01 Košice

Registr. zn. 787/93

For basic information about the journal see
Internet home pages: www.uvm.sk

Indexed and abstracted
in AGRIS, CAB Abstracts

FOLIA VETERINARIA, 48, 1, Supplementum, 2004

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FOLIA VETERINARIA

PUBLISHED BY
THE UNIVERSITY OF VETERINARY MEDICINE
KOŠICE, THE SLOVAK REPUBLIC

Folia veterinaria
Vol. 48, No. 1, Supplementum, 2004

VYDALA UNIVERZITA VETERINÁRSKEHO LEKÁRSTVA
KOŠICE 2004

University of Veterinary Medicine in Košice

Research report on the most important results obtained at the University of Veterinary Medicine in Košice in the period 2000—2003

Folia Veterinaria 48, 1, Supplementum, XLVIII — 2004

FOLIA VETERINARIA
www.uvm.sk

FOLIA VETERINARIA, 48, 1, Supplementum: S5—S6, 2004

RESEARCH ACTIVITIES OF OUR ANNIVERSARY-CELEBRATING UNIVERSITY AT THE ACCESSION OF THE SLOVAK REPUBLIC INTO THE EUROPEAN UNION

Introductory word of the rector of UVM and Chairman of the Advisory board of FOLIA VETERINARIA to the "*Research report on the most important results obtained at the University of Veterinary Medicine in Košice in the period of 2000–2003*"

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The University of Veterinary Medicine (UVM) in Košice was founded in 1949 and is the only university of its kind in the Slovak Republic. The aim of this institution is to prepare veterinarians and experts for the needs of veterinary practice. Another aim of this university is to deal with professional and scientific problems related to veterinary medicine on the level of the current knowledge.

At present, 728 students study at UVM in two study branches: *general veterinary medicine* and *hygiene of food*. After graduation, they are awarded the title MVDr. – doctor of veterinary medicine. In response to the needs of practice, bachelor study in the study branch *cynologist* has been initiated starting from the academic year 2004/05. In 1991 the University extended its activities by offering education for self-payers from abroad in English language. As a result of this, in addition to native students, veterinary medicine is studied at UVM by 123 students from Canada, Cyprus, France, Germany, Greece, Malta, Norway, Israel, Switzerland and United Kingdom. The international atmosphere at our university allows our students not only to gain professional knowledge but also, together with teachers, become enriched by multicultural influences in this creative environment. I am proud to state that in 1997 our university was evaluated favourably by the Evaluation Commission of EU with the conclusion that it complies with the European standards set in the EU for this type of university.

In addition to education of students, the scientific-research is an inseparable part of activities of our university staff. The research is oriented on four basic areas:

i) non-infectious diseases of farm animals (ethiopathogenesis, diagnostics, therapy and prevention) from the point of view of factors affecting their productive-reproductive abilities, surgical-orthopaedical diseases, the influence of external environment on internal and external indices of farm and free-living animals, fish and bees as well the influence of microbiotics and acidogenic substances on the occurrence and pathogenesis of diarrhoeal diseases of the young;

ii) infectious and parasitic diseases of farm animals and other animals of interest (ethiopathogenesis, diagnostics, therapy and prevention) with stress on immuno-diagnostics, the use of molecular-genetic methods in innovation of laboratory diagnostics of infectious diseases, more detailed study of the nature of pathogens and mechanism of their effect on the cellular level in some infectious and neoplastic diseases, study of relevant zoonoses and the effect of selected antiparasitic substances;

iii) management and protection of the environment of animals and humans focusing on the study of possible treatment and utilisation of organic wastes from animal production and processing plants, interaction of risk and essential elements in animals in risk areas, determination of the degree of contamination with xenobiotics and the degree of genotoxic damage to the organisms;

iv) hygiene, production and processing of safe foods, concentrating predominantly on the study of levels and properties of nitrosoamines, biogenic amines and products of lipid oxidation as well as procedures and methods for decreasing the presence of risk factors in milk and milk products in relation to consumer health. The research is also directed towards the hygiene of the environment in order to prevent production of harmful food of animal origin, occurrence of residua of pharmacologically active substances in food and raw materials of animal origin and on their possible elimination from the food chain. It also seeks potential ways how to affect the quality and health safety of meat products through the use of antioxidants during the fattening.

The above mentioned indicates that the research priorities of our institution are directed particularly towards areas stressed by the 6th Frame programme of the EU. We believe that this will contribute to more effective international collaboration between our university and universities in other Member states.

The scientific research at our university is carried out by 200 professionals from 7 departments and 4 clinics. The research teams are composed of 32 professors, 35 assoc. pro-

fessors, 89 assistants and 44 scientists. An important source for financing the research are grants VEGA SR, which are obtained on the basis of a state-wide competition. Part of the financial means was obtained through state programmes and state orders. At present, the university staff is involved in 65 grants VEGA, 4 state programmes and 2 state orders. Besides national sources, our staff submits regularly applications for international projects. We have been successful in obtaining projects based on bilateral collaboration within governmental programmes involving Greece, Hungary, Austria and Germany. Active collaboration has been established with institutions abroad, namely in Austria, the Czech Republic, Italy, Poland, Russia, Spain, Sweden, Ukraine and United Kingdom. The level of our scientific activities is witnessed by our participation in three projects within the 5th Frame programme of the EU and two projects within the 6th FP EU.

Our scientific-education activities are also directed towards the education of young scientists. This education starts even in the pre-graduate phase of university study during which the best students are engaged in the study of partial scientific problems under the guidance of experienced professionals. The scientific activities of the students culminate in the presentation of projects at university level in the form of student's scientific papers, frequently with participation of students from abroad. Students are motivated for this activities also by valuable prizes. One year ago the university representatives and its scientific board made a decision that the university study will be completed not only by state exams but also by diploma thesis underlain by an experimental scientific study. This should also contribute to increasing the scientific awareness of students.

An important way of upgrading the scientific youth is the exchange programme SOCRATES which permits exchange of pre-graduate students and teachers. This form was used by students who spent some time at universities in Bristol, Gent, Hannover, Helsinki, León, Lisbon, London, Lyon, Madrid, Milan, Padova, Perúgia, Thessaloniki, Utrecht, Vienna and Vila Real. The programme CEEPUS allowed other students to travel to Brno, Budapest, Ljubljana, Zagreb and Vienna.

Our PhD students, 24 at the present and of that 5 from abroad (Jemen, Yugoslavia, India, Vietnam), are involved actively in the work on scientific projects. A number of them have participated also in the international mobility programme SOCRATES. They are able to travel to scientific laboratories in different places of Europe and the world, obtain valuable experience and perform experiments which, with regard to our scientific equipment, could not be conducted at the present. Our aim is to make the graduate studies of PhD students and scientists from our university at well-known European and overseas universities a regular part of their scientific career. These studies will allow them not only to obtain new experience but form a good basis for establishing informal contacts for further collaboration within scientific projects.

The knowledge obtained during the work on scientific projects is presented regularly on national and international conferences, seminars and meetings. We have a long tradition of organising some international conferences at the premises of our university. The results of scientific studies are published not only in national but increasingly also in international scientific journals included in the Current Contents database.

Since 1956 our university has published a scientific journal FOLIA VETERINARIA. First it was published in Slovak but since 1993 all papers have been published exclusively in English. In this way we try to ensure that the published papers are accessible to the widest possible scientific community. Our journal is distributed to 41 countries round the world. It is gratifying that the number of contributions from foreign authors including the EU Member states has been increasing continuously.

In addition to four regular annual issues of this journal the Editorial office of FOLIA VETERINARIA issues occasionally a Supplementum devoted to a selected theme. The present Supplementum was prepared on the occasion of the 55th anniversary of our university and admission of the Slovak Republic to the EU. The Supplementum has been prepared as a Research Report. It focuses on presentation of projects in which our members of staff participated in the period of 2000—2003. It is a brief summary of our research activities in this period. For better understanding we should stress that this was the period during which the entire Slovak society had to face not insignificant economic problems associated with the rebuilding of the economy of our young republic. Despite this fact we made every effort to increase the qualitative level of scientific research at our university.

Individual projects are presented in the Supplementum in the form of short abstracts focusing on the results. Contact e-mail addresses of responsible persons should contribute to rapid communication with those who are interested in details, in obtaining copies of papers, establishing collaboration, etc. In order to obtain better picture, individual projects are presented in the following sequence:

a) basic disciplines,
b) infectious and parasitic diseases,
c) environmental problems and
d) hygiene of food, particularly of food of animal origin.

The results of several projects were obtained within international collaboration between departments of our university and European scientific institutions. Figuratively, we entered Europe in advance of the official political decisions of respective state bodies and volition of Slovak people. In conclusion I would like to express my hopes that this Supplementum of our journal FOLIA VETERINARIA will provide a more detailed picture of the research level at our university and will contribute at the same time to establishing new collaboration and intensifying the existing collaboration with our project teams, for example within the 6th Frame programme of the EU. Certainly, the science recognises no borders and should not focus only on explaining the laws of nature but also on unifying people and increasing the quality of their life.

Dr.h.c. Prof. Rudolf Cabadaj, DVM, PhD.
Rector of UVM and Chairman of the Advisory board
of FOLIA VETERINARIA

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THE QUALITATIVE EVALUATION OF FEED IN RELATION TO NUTRITIVE PHYSIOLOGY WITH IMPACT ON THE METABOLISM, PRODUCTION AND HEALTH OF MONOGASTRIC ANIMALS

Project VEGA SR, No. 1/9268/02: Duration of the project: from 01/2002 to 12/2004, principal investigator: Ľ. Bindas, DVM, PhD, Department of Animal Nutrition, Dietetics and Animal Breeding of UVM Košice

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The effect of acidifiers as an alternative to antibiotic growth promotors on digestion, metabolism and production was studied with the aim of influencing the performance and health of swine and poultry.

Changes in stomach pH were observed in three fistulated weaned piglets (Slovak White) five to eight weeks old, fed untreated rations, rations supplemented with 1.5 % calcium formate (CaF) or 1.2 % formic acid (FA) with the aim of studying digestive processes and manipulating them successfully. Samples of stomach contents were taken before feeding and after feeding at one hour intervals for six hours. The pH values measured one hour after feeding were significantly higher (control – 3.60, CaF – 3.30, FA – 2.62) in comparison with the pre-feeding level (1.53, 1.73, 1.40, resp.). Formic acid decreased the stomach pH more effectively as levels below 3 were reached as early as one hour after feeding while piglets from the CaF group exhibited such levels three hours after feeding (1, 2).

The effect of addition of 1.5 % CaF and 2 % Zeoform (Z – formic acid, CaF and zeolite) to complete mixed feed on the fermentation process in the digestive tract was investigated by examining the faeces of nine weaned piglets (Slovak White) divided into three groups. The highest concentration of VFA (63.02 g.kg^{-1} dry matter DM) was observed in the CaF group at the age of six weeks. The difference in comparison with the other groups was small and varied between 8—11 %. The concentration of acetic acid was the highest in all experimental groups (acetic acid 36—38 %, lactic acid 25—31 %, propionic acid 16—19 %, butyric acid 8—11 %, valeric acid 7—8 %). The difference in the pH of faeces between the groups was insignificant and ranged from 6.07 to 6.41 with the lowest values determined in the control and the highest in the CaF group. The highest levels of ammonia and nitrogen were observed in the Z group (Z: NH_3 — 534 mg.kg^{-1} DM, N 283 g.kg^{-1} DM; control group: NH_3 — 375 mg.kg^{-1} DM, N 271 g.kg^{-1} DM; CaF group: NH3 — 355 mg.kg^{-1} DM, N 255 g.kg^{-1} DM). (3)

The effect of supplementation of complete mixed feeds HYD-01, HYD-02 and HYD-03 with Zeoform (1 %) on performance, health, blood serum metabolic variables, and the level of nutrients in the large intestine and droppings was studied in 100 broiler chickens (COBB). Production variables of both groups (Z and the control) were comparable. No significant differences were observed in the blood serum parameters in both groups except for lower levels of uric acid in the Z group (285.9 µmol.l^{-1}) in comparison with control one (349.49 µmol.l^{-1}). The levels of ammonia and nitrogen in droppings and the large intestine content were lower in the Z group (4).

Performance, metabolic variables, ileal digestibility and some variables of caecum content (DM, pH, VFA, lactic acid, enterococci and lactobacilli counts) were studied in fifty broi- ler chickens (HYBRO) fed complete mixed feed supplemented with Z (1 %, no antibiotics) or antibiotics (control). Performance parameters did not differ significantly between the groups. A lower concentration of serum uric acid (3104 µmol.l^{-1}, control — 337.5 µmol.l^{-1}), higher ileal digestibility of nitrogen ($P<0.05$), a tendency to higher DM, pH, and lower values of propionic, lactic and total sum of fatty acids as well as enterococci and lactobacilli counts in caecum content and lower nitrogen (–14,1 %) and ammonia (–7.8 %) levels in droppings were observed in the Z group.

The comparison of the effect of CaF (0.6 %) and Z (1 %) supplemented to the complete mixed feed, based on plant protein sources, on the performance and some metabolic parameters showed higher weight gains and feed efficiency as well as the tendency towards decreased serum uric acid levels in broiler chickens fed complete mixture supplemented with Z (5).

Orientation of research in the near future: To develop methods and ways of assessment of the interaction between nutrition and protein and energy metabolism; to investigate the potential use of diaminopimelic acid in the process of evaluation of fermentation processes and nutrient digestibility in the digestive tract of monogastric animals.

1. Bindas, Ľ., 2002: Current trends in nutrition related to critical points affecting the health and productivity of pigs (In Slovak). *Slov. vet. čas.,* 27, 9—12.

2. Bindas, Ľ., Vajda, V., Maskaľová, I., 2002: The influence of acidifying substances on physiology of digestion in pigs (In

Slovak). *Proc. 5th Days of Nutrition and Veterinary Dietetics,* Hrádok, 169—173.

3. Bindas, L., Vajda V., 2003: The influence of acidifying substances and zeolite on productive parameters of fattening pigs (In Slovak). *Proc. 5th Kábrt's Dietetic Days,* Brno 2003, 1—5.

4. Demeterová, M., 2003: Acidifying substances – one of the alternatives to antibiotic growth stimulators in poultry (In Slovak). *Proc. 5th Kábrt's Dietetic Days,* Brno 2003, 11—15.

5. Demeterová, M., Vajda, V., 2002: Comparison of the effect of calcium formate and Zeoform on production and some parameters of the internal environment of broiler chicks (In Slovak). *Proc. 5th Days of Nutrition and Veterinary Dietetics,* Hrádok, 246—249.

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FOOD INTAKE CONTROL AND ENZYME ACTIVITY OF THE INTESTINAL TRACT IN ANIMALS

Projects VEGA SR, No. 2/6108/99, No. 2/2039/22: Duration of the projects: from 01/1999 to 12/2001, from 01/2002 to 12/2004, principal investigator: Š. Mozeš, DVM, PhD, Institute of Animal Physiology, Slovak Academy of Sciences

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The laboratories of physiological regulations have focused on the basic research related to the food intake and growth of animals. Within these projects long-term investigations have concentrated particularly on the causes of disorders in physiological functions that result in characteristic changes in food intake, food utilization, growth rate, body mass and body fat volume. Besides obtaining basic knowledge, the results opened the possibilities for practical application in the optimalisation of nutrition leading to in increase of animal productivity (4) as well as in human medicine where they can help in selection for effective strategy in the prevention and treatment of obesity.

MSG (monosodium glutamate) administration increased the body fat content and Lee's index of obesity, but was without any significant effect on the food intake. In MSG- treated animals permanently increased intestinal alkaline phosphatase activity was recorded (1, 2, 3). This indicated that besides neurohormonal and metabolic disturbances, also the altered intestinal function may be a further key factor contributing to the development of the MSG-syndrome in adult animals. Therefore the investigation of intestinal enzyme activity may be important for the study of MSG-induced and another forms of obesity.

Orientation of research in the near future: Our studies will focus on obtaining new knowledge on the functional maturation of the small intestine and the possible influence on the latter specific interventions during milk intake period and consequent somatic manifestation in weanlings and in adults.

In the short term our attention will concentrate on the relationship between development of obesity induced by neonatal application of monosodium glutamate (MSG) and the small intestinal functions.

Quantification of enzymatic changes in the small intestine (*duodenum* and *jejunum*) will be carried out in separated individual enterocytes and also in tissue sections by the microdensitometer Vickers M 85a.

1. Mozeš, Š., Lenhardt, Ľ., Martinková, A., 2000: Alkaline phosphatase activity of duodenal enterocytes after neonatal administration of monosodium glutamate to rats. *Physiol. Res.,* 49, 269—277.

2. Martinková, A., Lenhardt, Ľ., Mozeš, Š., 2000: Effect of neonatal MSG treatment on day – night alkaline phosphatase activity in the rat duodenum. *Physiol. Res.,* 49, 339—345.

3. Raček, Ľ., Lenhardt, Ľ., Mozeš, Š., 2001: Effect of fasting and refeeding on duodenal alkaline phosphatase activity in monosodium glutamate obese rats. *Physiol. Res.,* 50, 365—372.

4. Lenhardt, Ľ., Mozeš, Š., 2003: Morphological and functional changes of the small intestine in growth-stunted broilers. *Acta Vet. Brno,* 72, 353—358.

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DETERMINATION OF REFERENCE VALUES OF EQUINE HOMEOSTASIS AND THEIR RESPONSE TO STRESS FACTORS

Project VEGA SR, No. 1/7024/20, Duration of the project: from 01/2003 to 12/2005, principal investigator: Assoc. Prof. J. Kušev, DVM, PhD, I Internal Clinic of UVM Košice

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The condition of horses and their performance depend on the quality of the internal environment of their bodies that reflects their health status. The internal environment status is reflected in the most important parameters of the metabolic complex. It reacts sensitively to all external and internal stimuli, particularly to various load factors resulting from exploitation of horses. Our observations of the equine metabolic complex and its responses to the action of stress factors allowed us to draw important conclusions.

The following biochemical parameters were selected as most important for investigation of the metabolic complex in horses: AST, ALP, LDH, GMT, creatinine (Creat), AP, urea, total bilirubin (Bil. T), glucose (Glu), cholesterol (Chol), total lipides (Tot. lip), Ca, P and Mg (1).

We determined the reference values of TB, Glu, Chol, Urea, ALP, AST, LDH, GMT, Ca, P in the Slovak warm-blooded and Norik breeds.

Observations of the response of selected biochemical parameters to the age of horses showed:

– a decrease with age in ALP, Creat, Glu, Chol, LDH, Tot. lip, P,

– an increase with age in urea, TP and Mg.

Significant differences in the values of TP, urea, ALP, AST, LDH and Ca were observed between Slovak warm-blooded, English thoroughbred and Hutsul breeds (5).

Observations related to the influence of sexes showed differences in the level of urea and activity of LDH.

From the potential stress factors we selected stress related to riding to varying distances at different speed. We performed observations with total riding distance of 2400 metres and running time of ten minutes according to the following scheme: 1000 metres at a speed of 3.3 $m.s^{-1}$, 600 metres: 5.0 $m.s^{-1}$, 200 metres: 3.3 $m.s^{-1}$ and 600 metres: 5.0 $m.s^{-1}$ (3).

The running load was reflected in the values of the following biochemical parameters: AST and ALT showed no marked post-load increase; TP values increased; glucose showed an immediate marked decrease; Ca and P increased slightly; Mg levels varied (4).

Vitamin E was applied to horses *per os* and *i.m.* to eliminate the stress effects of physical load related to riding.

After application of vitamin E and the subsequent physical load the following results were obtained:

Application of vitamin E *per os (p.o.)* at a standstill decreased activity of AST while the exercise caused an increase in AST. The *i.m.* application of vitamin E increased activity of ALP which was not affected by the load. Application of vitamin E failed to affect GMT activity. The load itself as well as load after *i.m.* application of vitamin E decreased GMT activity. Neither application of vitamin E nor the exercise load affected the level of TP and cholesterol. The content of urea decreased after the load on day of *i.m.* application of vitamin E. The load on day of *p.o.* and *i.m.* application of vitamin E increased glucose level in horse blood while the load itself caused its decrease. Both the *p.o.* and *i.m.* applications of vitamin E as well as the load increased the level of P. The *p.o.* and *i.m.* applications of vitamin E increased the level of Ca while the load alone decreased the level of this element. The *p.o.* application of vitamin E increased the Mg level. However, combinations of vitamin E application and the exercise load resulted in a decrease in Mg levels (5, 2).

Orientation of research in the near future: It appears desirable to focus further research on looking for and testing effective anti-stressors and harmonisers of homeostasis to prevent homeostatic imbalances and increase the exploitation performance of horses.

1. Boldižár, M., 2003: *Dynamics of Levels of Some Vitamins, Enzymes and Minerals in Horses Blood* (In Slovak), PhD Thesis (tutor: Kušev, J.). UVM Košice, 55 pp.

2. Kasenčák, M.: *Levels of Some Vitamins and Enzymes under Various Physiological and Pathological Conditions* (In Slovak). PhD Thesis (tutor: Kušev, J.). UVM Košice, 56 pp.

3. Zvara, M., 2002: *Observation of Rest, Load, and Vitamin E on Selected Biochemical and Haematological Changes in Horse Blood* (In Slovak). Diploma Thesis (tutor: Kušev, J.), UVM Košice, 59 pp.

4. Boldižár, M., Kasenčák, M., Kušev, J., Weissová, T., Tučková, M., Nagy, O., 2002: Hypermagnesaemia of horses in the agglomeration of Jelšava-Lubeník (In Slovak). *XI International Symposium on Selected Aglomerations of Jelšava-Lubenik and Middle Spiš,* 144—148.

5. Boldižár, M., Kušev, J., Páleník, Ľ., 2002: Some fingings about confrontation of enviroment and quality of homeostasis (In Slovak). Miscellany *„Ecology and Veterinary Medicine“,* Košice, September 18–19, 2002, 51—52.

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THE STUDY OF HYPOTHALAMIC-HYPOPHYSEAL-OVARIAN RELATIONSHIPS IN NORMAL AND PATHOLOGICAL SEXUAL CYCLES

Project VEGA SR, No. 1/8039/01: Duration of project: from 01/2001 to 12/2003, principal investigator: Prof. J. Kačmárik, DVM, PhD, Clinic of Obstetrics, Gynaecology and Andrology of UVM Košice

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Our observations showed that 12-day administration of synthetic progestins to ewes with physiologically regular reproductive activity failed to affect the hormonal pituitary-ovarian status regarding both synchronised oestrus and metaoestrus. Combination of hormonal synchronisation of oestrus with subsequent stimulation by exogenic hypophyseal and extra-hypophyseal hormones induces extraordinarily high endocrine and histo-morphological activity of ovaries during oestrus and metaoestrus which affects negatively the processes associated with implantation of conceptus (1, 2).

The study of the effect of folliclegenesis-stimulating hormones allowed us to observe and confirm that the maturation of follicles, their ovulation and subsequent luteinisation depend on a number of factors, including those which affect the activity of leukocytes and angiogenesis. The growth, development and function of yellow corpuscles after biotechnical interventions seem to be just as important as the synchronisation effect itself (4).

The number, size and structure of Leydig cells in boars with various forms of cryptorchism differ markedly. The absolute number, proportion, and structure of Leydig cells in abdominal unilateral cryptorchid boars which lack the scrotal testis point to the stimulation of Leydig cells. The absolute number, size and proportion of Leydig cells in the abdominal testis of unilateral cryptorchids which have the scrotal testis are reduced considerably. The above mentioned observations, together with the structure of Leydig cells in TEM, indicate marked inhibition of hormonal activity of Leydig cells of intraabdominal testis. These results throw some light on the controversial literary data about structure and hormonal activity of Leydig cells in cryptorchids (3, 4).

Deficiency of zinc in boars causes degenerative changes in spermiogenic cells after meiosis and their depletion which results in the atrophy of seminiferous tubules and damage to Sertoli and Leydig cells. A deficit in zinc was accompanied by anorexia, growth disorders, and paraketosis. A single parenteral administration of preparation Zindep, inj. a.u.v., at a dose of 0.2 mg $Zn.kg^{-1}$ body weight, caused partial or complete recovery of spermiogenesis by day 20 and 60 after the administration, resp. We observed good tolerance and the high biological effectiveness of this preparation in terms of recovery of spermiogenesis and its favourable effect on the skin. Zinc as an essential element is inevitable for the normal course of reproductive functions in males (5).

Orientation of research in the near future: The aim of the second stage of the project is to observe the relationship between ependymal epithelium of cerebral ventricles including *eminentia mediana* and *plexus chorioideus* after hormonal induction of the sexual cycle in ewes and sows. Observations of the autocrine and paracrine mechanisms in follicle maturation will allow us to assess the ultrastructural and immunohistological changes in ovaries, oviducts, and the uterus.

In the second stage, after determining the optimum levels of copper and zinc, which can ensure normal reproductive functions in males, measures for prevention of infertility in boars will be proposed in the endangered territories.

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FOLIA VETERINARIA, 48, 1, Supplementum: S12—S13, 2004

CLINICAL EXPERIMENTAL OBSERVATIONS OF THE PATHOLOGY OF SOME DISEASE AND THE POSSIBILITIES OF INFLUENCING THEM IN THE SURGERY AND ORTHOPAEDICS OF MAMMALS, BIRDS AND REPTILES

Project VEGA SR, No. 1/7039/20: Duration of project: from 01/2000 to 12/2002, principal investigator: Prof. V. Ledecký, DVM, PhD, Clinic of Surgery, Orthopaedics and Radiology of UVM Košice

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It has been observed that the incidence of dental fractures culminates in young dogs up to the third year of age. The most affected are the frontal teeth as a consequence of increased irritability in young dogs and/or the use of inadequate training devices. Increased occurrence of caudal teeth fractures in older dogs is related to the function of the respective sectorial teeth. The degree of traumatisation of teeth located more caudally in the dental cavity is synergic with prognosis of preservation of vitality of dental pulp even after its longer exposure to the external environment. We observed a positive effect of corticoid flumetazone on the healing of dental pulp after its vital amputation (3).

Reconstruction of the anterior eye segment was carried out in rabbits using corneal grafting with parallel autotransplantation of the amniotic membrane. Clinical examination showed that eight out of fifteen operated eyes exhibited a very slight oedema in both donor and recipient zones which disappeared by the fourth week after surgery. Application of cyclosporine was discontinued after two months. Two weeks later three cases exhibited neovascularisation of the donor cornea. Erosions were observed after three months in four eyes and perforation of cornea in two eyes after nine months. Stability of the precorneal lacrimal film improved towards the end of observation and only three out of eight eyes (three weeks after the surgery) lacked continuous precorneal lacrimal film nine months later. The rabbits were killed after nine months and the corneas were sent for histopathological examination. This examination detected various stages of immune reaction in the refected donor cornea (five eyes) manifested by thinning of corneal stroma and infiltration with lymphocytes. The changes in corneal epithelium were most marked at the donor/recipient interface (4).

We conducted studies on 48 samples of keratin with drawn from 44 horses. In samples from four horses we examined separately the pigmented and non-pigmented keratin. Of the total horses examined 27 were mares, 3 stallions and 14 geldings. The interbreed differences and differences between samples from different breeding establishments were deterrmined by means of the Kruskal-Wallis non-parametric ANOVA test, and the differences between sexes by means of the Mann-Whitney test.

Statistical evaluation showed significant interbreed differences for zinc ($P<0.0013$) and calcium ($P<0.0067$) (5).

We evaluated some parameters of synovial fluid of twenty dogs with unilateral rupture of cranial cruciate ligament (RCCL). The total number of nucleic cells was < 3,000 cells. dl^{-1} in 17 out of 20 samples and exceeded 4,500 in three samples. The total count of cell elements (c. e.) in synovial fluid of undamaged joints ranges between 0 and 3,000 cells. dl^{-1}. The c. e. in dogs with traumatic or degenerative diseases do not exceed 5,000 cells.dl^{-1}. According to this criterium we may assume that in the 17 cases the c. e. of synovial fluid did not indicate inflammatory-degenerative processes. Total protein concentration in individual samples ranged from 2.0 to 4.2 g.dl^{-1}. with the mean value of 2.9 g.dl^{-1} which indicated a slight inflammatory process. On the basis of the parameters of synovial fluid determined in our study for RCCL joints we can conclude that except for indication of slight degenerative and inflammatory changes the synovia of examined joints diffred only little from that of healthy joints (1).

Experiments were conducted to evaluate the status of endogastric mucosa in dogs after administration of ketoprofen tablets. The experiment lasted five weeks. The preparation itself was administred for four weeks. The drug was administered for 21 days at a dose of 1 mg.kg^{-1} b. w. and for another week at a dose of 0.5 mg.kg^{-1} b. w. No preparation was administered during the fifth week when the rate of healing of lesions induced in the gastric mucosa was observed. Gastroscopical examination of dogs took place before the experiment and after weeks 1, 2, 3, 4 and 5 of its duration. Lesions in endogastric

mucosa were observed in all dogs already seven days after the onset of ketoprofen administration. All of them were moderate. The changes increased with the time of administration of the preparation. After administering half-dose of ketoprofen (fourth week of the experiment) the endogastric mucosa lesions started to recede. After termination of its administration (days 28—35) the lesions started to heal rapidly (2).

In the experimental stage of the project we compared the therapeutic effect of various methods of palliative and surgical interventions in green iguana with diagnosed ileum. We developed a therapeutic protocol for two different palliative therapeutic approaches and two surgical ones with different post-operative therapy and compared the therapeutic effect.

Orientation of research in the near future: Our investigations will be directed according to the following requirements: the use of cementate endoprosthesis of coxal joints in the therapy of hip joint dysplasia in dogs. Therapy of bone cartillage defects using methods of tissue engineering. Comparative studies of evaluation of hip joint dysplasia using FCI-approved and Penn-HIP methods. Investigation of the possibility of affecting keratin in horses which serves as an indicator of their mineral metabolism. Traumatic wounds in free living birds and observation of their effects on their subsequent reintroduction. Published results can be obtained from the author.

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FOLIA VETERINARIA, 48, 1, Suplementum: S14—S15, 2004

THE DEVELOPMENT OF NEW FORMS OF SUPPLEMENTATION OF COPPER AND IODINE TO ANIMALS IN RESPECT OF THEIR METABOLIC INTERACTIONS

Project VEGA SR, No. 1/7034/20: Duration of project: from 01/2000 to 12/2002, principal investigator: Prof. J. Bíreš, DVM, DSc, II Internal Clinic of UVM Košice

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The project investigated metabolic disorders of the microelements Cu, I, Fe and Zn from the point of view of their frequency, etiology, diagnosis, prevention and therapy in ruminants and pigs. The most endangered animals with regard to metabolic disorders of the trace metals studied are the young, mothers in an advanced stage of pregnancy and highly productive animals kept in regions exposed to industrial emissions.

The etiology of metabolic disorders of Cu, I, Fe and Zn is polyfactorial. Optimal saturation of animals with these microelements is affected particularly by their uptake in feed, reabsorption processes, intermediary metabolism and excretion. The diagnosis of metabolic disorders of microelements should be based on the nutrition programme, productive orientation, reproductive phase of animals and analysis of specific markers of metabolism on the level of resorption, intermediary metabolism and excretion (2).

An injectable, copper-based preparation was developed for prevention and the treatment of deficiency of Cu or Fe. Its testing in ruminants and pigs confirmed its biological effectiveness and local and total tolerance. Potassium or sodium salts of iodine, orally supplemented to ruminants, effectively prevented the deficiency of iodine. The males of small ruminants and boars deficient in zinc exhibited histological changes in the seminiferous epithelium accompanied by spermiogenesis disorders.

Basic mechanisms of toxicity to biological functions of ruminants of risk elements present in magnesite fly ashes and emissions from plants producing copper, zinc and aluminium were described under experimental and breeding conditions. Experiments were conducted on the level of animal bodies involving laboratory diagnosis of metabolic disorders of Cu, Fe, and I using markers of haematological (Er, Hb, Hk, Lc) proteinaceous (CB, Alb, CIg), energetic (Glu, NEMK, TL, TCH, TG), hepatal (AST, ALT, GGT, ALP, T, bil.) and mineral profiles (serum concentrations of Cu, Fe, I, or elements entering the interaction with Zn and Se, or risk elements, such as Cd, Pb, As).

The study of the effect of injection supplementation of Cu on dairy cows suffering from hypochromic anaemia thirty days before parturition showed an increasing cupraemia in the treated cows up to day 4 post-application and significant differences in serum Cu levels between experimental and control groups ($P<0.05$; $P<0.01$) persisting up to day 15 after calving. Biological effectiveness of the preparation tested in pregnant cows with hypocupraemia, sideropenia and hypochromic anaemia was manifested by gradual adjustment of colour of hair and mucous membranes in the treated cows starting from day 20 after injecting the copper preparation (4).

We recorded the transplacentary and transmammary dependence of calves on injection supplementation of Cu to mothers during pregnancy. The biological effectiveness of the Cu-based preparation supporting the positive effect of Fe-containing preparation on haematopoiesis was confirmed (3).

The effectiveness of the oral supplementation of inorganic iodine salts was evident within three weeks in terms of the adjustment of the anatomical and functional mechanisms of the thyroid gland with a positive influence on the total metabolism.

It was observed that the phenomenon of the productive health of animals reared in the deposit area of Krompachy, Jelšava-Lubeník and Žiarska fold industrial plants is determined by undisturbed interactions of animals with their life needs, i.e. an optimum ratio of nutrient uptake and expenditure including toxic elements found in the regions round industrial plants, and participation of homeostatic regulation of the total metabolism.

Observations under experimental and breeding conditions showed that the toxic mechanisms of fly ash deposited in the central Spiš area, most important to the health and productivity of sheep, resulted in hepatotoxic, haemolytic, immunosuppressive, mutagenic, reproductive, and nutritive disorders (1).

Orientation of research in the near future: The project will focus on the study of basic physiological and pathological mechanisms in biologically critical stages, i.e. 3 to 2 weeks before parturition and up to ten or thirty days post-parturition, in dairy cows, sheep, goats and sows. Our observations will be directed twoards the nutritive, regulation, homeostatic, metabolic and immunologic relationships which participate in the physiological and pathological processes during pre-, peri-

and postnatal periods that may affect the health of mothers, the biological quality of colostrum and health of the young. The knowledge obtained will be used in the active control of health of mothers and the young, i.e. by means of predetermined regulation mechanisms and metabolic interactions, and in investigations of productive and reproductive health in the pre- and peripartal periods.

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FOLIA VETERINARIA, 48, 1, Supplementum: S16—S17, 2004

HEALTH AND MORBIDITY OF ANIMALS FROM THE POINT OF VIEW OF HOMEOSTASIS AND ITS DISTURBANCES CAUSED BY NEGATIVE ENVIRONMENTAL INFLUENCES – DIAGNOSIS, THERAPY AND PREVENTION

Project VEGA SR, No.1/7026/20: Duration of the project: from 01/2000 to 12/2002, principal investigator: Prof. G. Kováč, DVM, DSc, II Internal Clinic of UVM Košice

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The project allowed us to determine the factors of the external environment that were reflected in the level of parameters which indicated the disturbances of individual systems of animals. We used the following profiles: haematological, immunological, macro- and micromineral, energetic, protein, enzymatic, vitamin, ruminal, urinary, milk, etc. A number of diagnostic, therapeutic and preventive procedures was validated and applied in this direction (2).

Chromosomal analysis (CA), a test of sister chromatid exchanges (SCE) and micronuclei test (MN) in peripheral lymphocytes of cattle exposed to herbicide chloridazon were used to evaluate its genotoxicity. The action of chloridazon on the level of 7.10^{-4} mol resulted in increased damage to chromosomes reflected in all tests used (CA, MN, SCE) resulting from its direct effect on peripheral lymphocytes (important with regard to protection of animal health and increasing risk to humans through the food chain) (4).

The mutation damage induced (MN) by action of a hepatotoxic substance (CCl_4) in relation to application of protective agents, particularly antioxidants (vitamin E and selenium), to sheep *in vivo* was investigated. CCl_4 at a dose of 0.05 $mg.kg^{-1}$ b. w. increased significantly the frequency of MN in sheep. Vitamin E and selenium acted as protective substances and effective inhibitors of MN frequency in the peripheral lymphocytes of sheep *in vivo* (3).

The effect of long-term action of low and high doses of mycotoxin zearalenon on selected biochemical parameters in blood serum of animals was investigated. Significantly increased levels of dopamine, norepinephrine and epinephrine were observed in rabbits which were supplied zearalenon. This mycotoxin affects the levels of plasma catecholamines in rabbits most likely through estrogens or chemical stress. Zearalenon applied at a dose of 100 kg^{-1} b. w. caused an evident increase in enzyme activities of AST, ALT, ALP, GGT, and LD which suggested its hepatotoxic effect. Our biodegradative tests showed that yeasts *Saccharomyces* spp., appeared to be most effective towards decreasing the concentrations of zearalenon but also of other selected mycotoxins (1).

With regard to environmental hygiene Persteril was the most suitable and effective disinfectant. Natural sorbent zeolite may be used effectively in the treatment of wasters (decreasing nitrogen and phosphorus in the effluent) (2).

Orientation of research in the near future: Our future research will be concerned with the high density of high-yield animals under the current breeding conditions (stanchion and free housing, complete mixed feeds with regard to reproductive cycle) which places increased demands on optimisation of the entire process intended for obtaining products of animal origin. This complex is associated with a number of risk factors reflected in effective use of production potential of the animals, increased susceptibility to diseases, decreased quality of products, premature culling of individual animals and decreased economy of breeding. No less important is the deterioration of the environment with respect to the contamination of soil and water (surface, ground, atmospheric). This reality calls for development and application of suitable, modern methods for diagnosis, therapy and prevention of important disease units based on thorough knowledge of etiopathogenesis of diseases and studies conducted on cellular and subcellular levels. In this direction it appears necessary to analyse the problems contributing to decreasing quality of feed, e.g. by contamination with fungi, heavy metals or pesticides, and their immediate impact on disturbances of animal homeostasis.

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FOLIA VETERINARIA, 48, 1, Supplementum: S18—S19, 2004

THE IMMUNOMODULATIVE, CYTOTOXIC, AND GENOTOXIC EFFECT OF XENOBIOTICS ON EUKARYOTIC AND PROKARYOTIC CELLS AND VIRUSES

Project VEGA SR, No. 8115: Duration of the project: from 01/2000 to 12/2003, principal investigator: Prof. J. Pistl, DVM, PhD, Department of Microbiology and Immunology of UVM Košice

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Pesticides constitute a group of chemical substances extensively applied in nature. Consequently, the aim of the project was to evaluate the immunotoxic and genotoxic potential of selected pesticides with a different chemical structure and mechanism of action (atrazine, bentazone, chloridazone, dichlofluanid, endosulfan, MCPA, simazine and triallate) in Merino-sheep under *in vitro* conditions.

An iodo-nitro-tetrazolium reductase test (INT) was used to evaluate the effect of these pesticides on the metabolic activity of phagocytes. Determination and differentiation of the toxic and immunotoxic effects was carried out by the leukocyte migration-inhibition test (LMIA).

A significant decrease in metabolic activity of phagocytic cells isolated from sheep peripheral blood was observed after the exposure to a single pesticide when dichlofluanid (1.10^{-3}—1.10^{-6} mol.l^{-1}), endosulfan, simazine and triallate (1.10^{-3} mol.l^{-1}) were tested (1).

A cytotoxic effect (a significant decrease in spontaneous migration of indicator leukocyte cells) was recorded for bentazone, dichlofluanid, endosulfan and MCPA (1.10^{-3} mol.l^{-1}), chloridazone (1.10^{-3} mol.l^{-1}—1.10^{-4} mol.l^{-1}) and triallate (1.10^{-3}—1.10^{-7} mol.l^{-1}) (2).

An immunotoxic effect (a significant decrease in the lymphocyte response to mitogenic stimuli in LMIA) was revealed for the following pesticides: atrazine (1.10^{-3}—1.10^{-4} mol.l^{-1}); bentazone (1.10^{-4}—1.10^{-6} mol.l^{-1}); dichlofluanid (1.10^{-4}—1.10^{-5} mol.l^{-1}); endosulfan (1.10^{-4}—1.10^{-6} mol.l^{-1}) and simazine (1.10^{-3}—1.10^{-6} mol.l^{-1}) (2).

Three of the pesticides tested decreased both the metabolic activity of phagocytes and activation of lymphocytes with mitogen (dichlofluanid, endosulfan and simazin). Triallate showed a significant cytotoxic effect because it inhibited the metabolic activity of phagocytes as well as migration of indicator leukocytes in LMIA. The pesticides atrazine, bentazone and MCPA inhibited the mitogenic activation of lymphocytes only which suggested their immunotoxic effect (2).

The immunological functional tests showed a relationship between the chemical structure of the pesticides studied and the intensity of the influence on the function of phagocytes and lymphocytes in sheep peripheral blood. They also enabled the characterisation of the cytotoxic and immunotoxic effects of single chemical substances.

The genotoxic effects of pesticides (endosulfan and dichlofluanid) were evaluated in sheep peripheral blood lymphocytes under *in vitro* conditions by chromosome aberration and lymphocyte micronucleus assays (3). Endosulfan increased significantly the chromosome aberration frequency to 11 % (1.10^{-5}; 1.10^{-6} mol.l^{-1}) compared to 1.5 % in DMSO control and decreased the mitotic index at the concentrations of 1.10^{-5}— 1.10^{-7} mol.l^{-1}. At higher concentrations (1.10^{-5}—1.10^{-6} mol.l^{-1}) a significant increase in the number of micronuclei (64 micronuclei/1000 cells *versus* 23 micronuclei/1000 cells in DMSO control) was recorded. The results showed a weak genotoxic effect of endosulfan in sheep peripheral blood lymphocytes *in vitro* (4, 5).

Orientation of research in the near future: The main objectives include the study of the effect of xenobiotics with different chemical structure on the biological characteristics of viruses and of virus-cell interactions. Knowledge concerning the effect of subcytotoxic concentrations of xenobiotics with different chemical composition on the cells of the immune system and the organ tissues in relation to their sensitivity to virus infections is scarce. The studies will also focus on the influence of xenobiotics on the cells in different stage of viral infections under *in vivo* and *in vitro* conditions.

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FOLIA VETERINARIA, 48,1, Supplementum: S20, 2004

BIOTRANSFORMATION OF ENVIRONMENTAL CONTAMINANTS IN THE DIGESTIVE TRACT OF ANIMALS AND MECHANISMS OF SPREADING OF THE GENES RESPONSIBLE FOR THESE PROCESSES

Projects VEGA SR, No. 2/7026/20 and 2/3064/23: Duration of the projects: from 01/2000 to 12/2002, principal investigators: Prof. P. Javorský, RNDr, DSc, Department of Physiology of Farm Animals SAS and Prof. Ing. V. Lenártová, PhD, Department of Chemistry, Biology and Biochemistry of UVM Košice

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Micro-organisms have developed an effective protective mechanism for the elimination of toxic and mutagenic oxygen metabolites which involves the activation of antioxidant enzymes, e.g. superoxide dismutase (SOD, EC 1.15. 1.1), catalase (KAT, EC 1.11.1.6.), and glutathione-dependent enzymes of glutathione peroxidase (GSHPx, EC 1.11.1.9) and glutathione reductase (GR, EC 1.6.4.2).The response of these enzyme systems of rumen bacteria *S. bovis* 4/1 and *S. ruminantium* E32 to the environmental stress differed under aerobic and anaerobic conditions and also with respect to individual metals. With regard to *S. bovis,* manganese was a cofactor of SOD enzyme (Mn-SOD) while no SOD activity was observed in *S. ruminantium* regardless of the presence of metals. Mercury as a metal which reacts particularly with thiol groups increased the specific activity of Mn-SOD in *S. bovis* significantly. Activity of both GSHPx and GR increased under aerobic conditions. At the same time we observed a significant increase in the values of TBARSu, the biomarker of lipidic peroxidation, which indicates that the anti-oxidative enzymes failed to sufficiently protect the bacteria against the oxidative stress induced by mercury (1, 2).

Cu^{2+} a Cr^{6+} induce oxidative stress through production of reactive forms of oxygen *via* the Fenton reaction. Both these metals decreased the activity of Mn SOD in *S. bovis* significantly. Glutathione-dependent enzymes GSHPx and GR responded differently. Their activity increased or remained unchanged in both *S. bovis* and *S. ruminantium.* The values of TBARS increased similarly in both bacteria. The results indicate that Hg^{2+}, Cu^{2+} and Cr^{6+} caused oxidative stress but the mechanisms responsible for the resistance of bacteria to individual metals differed (3).

Also plant non-protein antioxidants, such as vitamins (A, C, E) and trace metals (Zn, Cu, Mn, Se) may contribute to the elimination of the oxidative stress factors. Our experiments confirmed an unambiguous effect of some plant antioxidants (Se-L-methionine, α-tocophenol, β-carotene) on the activity of anti-oxidative enzymes which participated in elimination of the environmental stress induced by mercury in rumen bacteria *S. bovis* 4/1(4).

Orientation of research in the near future: Plant antioxidants as well as the genes of resistance to heavy metals originating from soil bacteria, incorporated in rumen bacteria, may play an important role in the elimination of environmental stress on the microbial rumen ecosystem and in this way contribute to adaptation of ruminants to their respective environment. The aims of our future research are to test the susceptibility of rumen protozoa to $HgCl_2$ and $CuCl_2$, to study the activation/inhibition of antioxidant enzymes in the presence of heavy metals and to investigate the influence of plant antioxidants of non-protein character on the elimination of environmental stress caused by heavy metals in rumen bacteria and protozoa.

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FOLIA VETERINARIA, 48, 1, Supplementum: S21—S22, 2004

OBSERVATION OF THE INFLUENCE OF BIOLOGICALLY ACTIVE SUBSTANCES ON THE PRODUCTIVITY AND HEALTH OF POULTRY, DIAGNOSTICS, THERAPY AND PREVENTION OF ITS HEALTH DISORDERS

Projects VEGA SR, No.1/7036/20 and No. 1/0575/03: Duration of sthe projects: 1st – from: 01/2000 to 12/2002; 2nd – from 01/2003 to 12/2005, principal investigator: Assoc. Prof. J. Šály, DVM, PhD, I Internal Clinic of UVM Košice

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Our investigations focused on the influence of heating fish meal intended for chickens to temperatures at which the histidine present in the fish is converted to gizerosine. Chickens supplied feed containing 10 % of fish meal showed a decreased uptake of feed and retarded growth starting from week 4 of the experiment. Histopathological observations showed an extension of small intestine mucous villi, damage to kidneys even necrosis of cells, changes in hepatocytes, and decreased activity of alkaline phosphatase in the microvillous zone of small intestine and in the liver. Blood cholesterol and triglycerides decreased and proteins increased. Haematological parameters showed no significant changes. Mechanical properties of bones, such as thickness, fracture stress, toughness, rigidity, direction of growth of bone tissue, and cross section area of bones at fracture sites were affected. Interaction of the heated fish meal with lead added at a dose of 25 mg.kg^{-1} in the form of lead acetate resulted in a significant negative effect on weight gain, number of heterophilic granulocytes, values of erythrocytic indices MCHC and MCH, blood cholesterol level, growth of the compacta towards bone axis, some mechanical properties of bones and caused histopathological and histochemical changes in the small intestine, liver and kidneys (1,5).

Acute oral toxicity LD50 of sodium salinomycin, an anticoccidial and biostimulatory substance, contained in a new preparation Synvertas plv. a.u.v. (Biotika Slovenská Ľupča) was determined to be 102 mg.kg^{-1} body weight (1020 mg of the preparation) in two-week old chickens and 106 mg.kg^{-1} in four-week chickens with the upper threshold of reliability equal to 109.9 mg and the lower one 102.09 mg.kg^{-1} b.w. LD50 of white rats of the Wistar breeding was 29.44 mg.kg^{-1} b.w. (4)

A study of the subacute toxicity of sodium salinomycin was conducted by administering this substance to five-week old chickens for seven days through a probe to the crop at the doses of 53 and 106 mg.kg^{-1} b.w. The lower dose caused no clinical, haematological or biochemical changes in the blood of the chickens. The higher dose failed to induce clinical symptoms of intoxication but the body weight of chickens decreased significantly and their blood urea and cholesterol increased (3).

Subchronic toxicity observations consisted in the application of sodium salinomycin to chicks with feed for 42 days at doses of 60 mg, 90 mg, and 180 mg.kg^{-1} in the form of preparation Synvertas plv.a.u.v. and a dose of 60 mg.kg^{-1} in the form of preparation Sacox 120 (Hoest Roussel Vet.). Sodium salinomycin supplied at a dose of 60 mg as a preparation Synvertas plv. a.u.v. significantly increased the weight of chicks after 42 days in comparison with control layers and all other groups of chicks. The 90 mg dose of sodium salinomycin decreased the body weight of chickens. A more pronounced decrease was observed in chickens fed the 180 mg.kg^{-1} feed dose. This dose caused also a significant decrease in the number of erythrocytes and the content of haemoglobin and calcium and an increase in the blood bilirubin. *Post mortem* examination showed moderate hyperaemia and increased spleen in some chickens (2).

Attention was paid also to the problems related to necrotic enteritis in birds. With regard to the aetiology of this disease we recognise also involvement of alpha-and beta-toxins which cause toxaemia, necrotic changes, haemolysis and death. Antibiotics cannot be administered en bloc but according to the sensitivity of birds. Supplying water acidified with vinegar (3 litres per 100 litres of water) or acidified feed (propionic acid, formic acid) appeared effective.

Investigations were carried out on three farms to determine the relationship between supplementation of feed with vitamin A and the level of this vitamin in the liver and the body weight of chicks at the end of fattening. Higher level of vitamin A in feed resulted in a higher slaughter weight of chicks and vitamin A levels in the liver. The level of vitamin A in the liver was higher in chicks with healthy livers compared to those with pathologically changed livers.

Our investigations included observation of factors that cause haemorrhagias in chicken muscles. They include various intoxications, particularly with drugs and mycotoxins, avitaminoses (A and K), infections and parasitoses. Muscle haemorrhages arise particularly during culling, transport and slaughter when animals are exposed to exertion, stress, temperature variations, draught, electric current, etc.

Orientation of research in the near future: We will investigate the effect of various biologically active substances on the intensity and quality of productivity and health of poultry and focus our studies on the etiology, diagnostics, therapy and prevention of topical health disorders in poultry and birds. Our investigations will include clinical and pathological-morphological changes, productive, haematological and biochemical parameters using specialised examinations.

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FOLIA VETERINARIA, 48, 1, Supplementum: S23, 2004

BIOTHERAPY OF CHRONIC WOUNDS AND DECUBITI OF ANIMALS AND HUMANS BY FLY LARVAE AND BEE PRODUCTS

Project VEGA SR, No. 1/9016/02: Duration of the project: from 01/2001 to 12/2002, principal investigator: Assoc. Prof. A. Kočišová, DVM, PhD, Department of Infectious Diseases and Parasitology of UVM Košice

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Larvae of necrophagous flies *Lucilia sericata* and *Phormia regina* (Diptera: Calliphoridae) were used in an alternative treatment of chronic skin diseases and soft tissues of animals. The mean length of the developmental cycle of *Lucilia sericata* (12.5—14.1 days) and *Phormia regina* (12.03—13.5 days) was affected significantly by the environmental temperature. The relative humidity had no effect on the cycle length (3). The comparison of the effect of antibiotics and disinfectants used to ensure sterility of larvae showed that 1 % solution of peracetic acid (100 %) and 1 % solution of glutaraldehyde were the most effective and can be recommended for preparation of sterile larvae intended for alternative ways of treatment (2).Tests were performed under laboratory conditions using larvae of *Phormia regina* to treat breeding rabbits with chronic decubiti of hind extremities and in one case also with abscess of a knee joint (1). Larvae were applied at a ratio of 8—12 per 1 cm^2 of the wound according to the volume of the necrotic tissue. By day 3 after application of larvae, the majority of necrotic tissue had been removed and a gradual production of healthy granular tissue was observed that slowly overlaid the entire wound. Two applications were necessary to treat the knee joint but already at the first redressing a visible regression of the inflammatory process and purulent discharge could be observed (4).

Orientation of research in the near future: Our results of observations carried out under laboratory and clinical conditions will be used to develop an optimum, reliable, and legislatively supported biotherapy procedure using larvae of flies to treat chronic wounds, decubiti, and osteomyelitis in humans. In the field of veterinary medicine we intend to use the larvae of flies to eliminate abscesses, chronic wounds of cutis and subcutis and some types of malignant and benign tumours as an alternative to costly surgical procedures.

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FOLIA VETERINARIA, 48, 1, Supplementum: S24, 2004

THE TOXIC, GENOTOXIC, AND PHARMACOLOGICAL EFFECTS OF XENOBIOTICS ON ANIMALS

Project VEGA SR, No. 1/8022/01: Duration of the project: from 01/2000 to 12/2003, principal investigator:
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The project consists of four thematically interconnected tasks:

1. The use of tests for determination of the genotoxic properties (mutagenicity and cytotoxicity) of agrochemicals (pesticides and cyclic hydrocarbons) using chromosomal analysis (CA), micronuclei and sister chromatide exchanges (MN, SCE), and mitotic and proliferation indices (MI and PI) of xenobiotics (pesticides) on a molecular level to assess the risk to farm animals in relation to paragraphs 2) and 3). The published results see as title 1 and 2.

2. The study of the chronic effect of low doses of cyclic substances and keto-compounds on animal bodies, the mode of their action and risk assessment with regard to farm animals. Protective capacity of animals and the ability to defend themselves against oxidative stress is given namely by biotransformation-detoxication (cytochrome 450s monooxygenases). Another marker of such exposure of animals is the detoxication capacity of the target organs involving AST, ALT, and GMT enzymes.

3. The testing of antibiotics and other xenobiotics for induction (inhibition) of non-specific, drug-metabolising, microsome enzymes in parenchymatous organs with subsequent determination of their additional pharmacodynamic parameters and respective biological functions.

4. The isolation and purification of growth factors from biological sources and the study of the mechanism of the transfer of mitogenic signals mediated by these substances to defined cell cultures. Insulin-like growth factors (IGFs) are important in the regulation of normal growth and development of foetuses. Their action and bioactivity are modulated by IGF binding proteins (IGFBPs) and specific IGF-1R receptors (3, 4, and 5). The aim of this project part is to isolate the binding proteins from the amniotic fluid of ruminants which has been considered so far biological waste.

Orientation of research in the near future:

– Quantitative (Comet assay) and qualitative (FISH) evaluation of changes induced in the genetic material on the basis of the combination of fluorescence and DNA hybridisation techniques.

– The amniotic fluid will be sampled from sheep and possibly also from other animal species in various stages of gestation and variations in the content of IGFBs will be determined during gestation. The binding proteins will be identified in a molecular mass and isolated by the Western and ligand-blot methods.

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FOLIA VETERINARIA, 48, 1, Supplementum: S25, 2004

THE ASSESSMENT OF ENVIRONMENTAL GENOTOXICITY BY MEANS OF BIOMARKERS AND PCR TECHNIQUE

Project VEGA SR, No.1/8024/01: Duration of the project: from 01/2001 to 12/2003, principal investigator: Assoc. Prof. K. Šiviková, RNDr, PhD, Department of Pathological Anatomy, Pathological Physiology and Genetics of UVM Košice

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In vitro validation of genotoxicity of chemical agents has been carried out using conventional procedures. Primary attention has focused on chromosomal aberrations (CA) that have been related to mutagenic and carcinogenic action of the chemicals. Sister chromatid exchanges (SCE) induced by both genotoxic and non-genotoxic carcinogens have been considered very sensitive biomarkers for validation of genotoxicity of a large group of chemicals. Information about the frequency of induced micronuclei (MN), which is used to detect clastogenic and aneugenic effect of the chemicals, is a suitable bioindicator in genotoxicity testing.

Our experiments focused on the validation of the genotoxic effect of carbon tetrachloride (CCl_4) in ovine peripheral lymphocytes *in vivo* and *in vitro* and on obtaining the proof of protective action of antioxidants (selenium and vitamin E) against the induced genotoxicity (1). The presence of carbon tetrachloride has been detected also in the environment. It has been used as dilutents in various manufacturing processes.

A clear dose-related response suggesting induced chromosomal damage was observed after exposure of ovine peripheral lymphocytes to CCl_4 for 48 hours *in vitro* (2). Cell-cycle delay was documented by additional variables, e.g. a reduction of proliferation (PI) and mitotic (MI) indices. Parallel *in vitro* culturing with CCl_4, vitamin E and Se proved considerable protective action of the two antioxidants reflected in a decreased chromosomal damage in comparison with cultures that contained carbon tetrachloride without any protective substances. A similar protective effect of antioxidants was observed also *in vivo* in a group of animals administered CCl_4, vitamin E and selenium (3). The results presented confirmed the sensitivity of ruminants to the chemical investigated and called attention to a potential increased genetic risk mediated by the food chain.

Besides testing the genotoxic action of CCl_4, we made an attempt to detect the presence of enterotoxins and enterotoxigenic staphylococci strains in raw sheep milk and other food. Immunochemical methods (RIA, ELISA) were used to determine the quantity of produced enterotoxin and molecular-genetic methods (PCR) were employed to detect the presence of toxin genes. Genes coded for staphylococci enterotoxins were detected in *S. aureus* isolates obtained from samples of food and swabs from technological equipment in food producing facilities. The highest proportion of toxin-producing isolates was found in sheep cheese (47.4 %) and pasta (33.3 %) (4). Our results indicate that detection of enterotoxins or enterotoxigenic strains in food may become an important tool in protection of consumers against staphylococcal enterotoxicosis.

Orientation of research in the near future: We plan to focus on the development of molecular methods, particularly fluorescent *in situ* hybridisation (FISH), which will complete the conventional cytogenetic methods currently used to evaluate the genotoxic potential of chemical substances. The FISH method is relatively new and is used in genotoxic studies to detect stabile chromosomal aberrations of the rearrangement type. Up to this time this technique has been used successfully in human clinical cytogenetics and prenatal diagnosis to detect chromosomal aberrations associated with birth defects. With regard to veterinary diagnostics, the FISH technique may become a useful endpoint capable of detecting balanced structural aberrations such as translocations that occur frequently in cattle herds and have negative effects on fertility of the carriers.

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FOLIA VETERINARIA, 48, 1, Supplementum: S26, 2004

OBSERVATION OF THE OCCURRENCE OF *foramen ovale patens* IN THE BREEDS AND LINES OF SLAUGHTER PIGS

Project VEGA SR, No. 1/9015/02: Duration of the project: from 01/2002 to 12/2004, principal investigator:
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We investigated the occurrence of foramen ovale patens by examining 96 hearts of pigs of the Slovak meat (SM) breed, 38 hearts of the Yorkshire breed (YO), 51 hearts of Slovak meat x Yorkshire (SM x YO), 43 hearts of Pietrain x Yorkshire (PN x YO) and 45 hearts of Pietrain x Slovak meat (PN x SM) breed at a pig fattening station.

Foramen ovale patens was observed only in seven pigs of the Slovak meat breed (SM) which corresponds to 13.7 % occurrence and in four pigs of Pietrain x Slovak meat breed (PN x SM), i.e. 11.2 %. Foramen ovale patens was not recorded in the other breeds examined. All positive cases of the Slovak meat breed were males. In case of Pietrain x Slovak meat breed positive signs were detected in two males and two females (1).

With regard to the size of foramen ovale patens in pigs of Slovak meat breed, 2 mm defects were observed in four pigs and 3 mm in three pigs. In the breed Pietrain x Slovak meat none of the defects were bigger than 2 mm. Confrontation of results obtained in this study with those from our previous observations called for additional studies devoted to the occurrence of this heart defect on the level of lines of individual breeds of slaughter pigs (2).

Observations on the level of lines allowed us to detect this defect in Slovak Large White (SLW) — 6, Landrace (LA) — 4, SM — 3 and PN x SM — 4. The following productivity and slaughter value parameters were determined and evaluated at the respective fattening establishment: mean daily weight gain, length of the slaughter trunk in cm, cross section area of *musculus longissimus dorsi* in cm^2, percentage proportion of thigh meat of slaughter halves and bacon height.

The highest daily weight gains were recorded in Yorkshire pigs (0.907 kg) and the lowest in those of Pietrain breed (0.742 kg). The longest slaughter trunk was found in Landrace pigs (80.44 cm) and the shortest in the Pietrain breed (75.95 cm). The biggest area of *m. longissimus dorsi* was observed in Pietrain (59.66 cm^2) and Yorkshire (56.31 cm^2) breeds. Pigs of these breeds showed also the highest percentage of thigh meat of slaughter halves (Pietrain — 24.26 %, Yorkshire — 22.39 %). Maximum bacon height was found in Slovak Large White (1.98 cm) and the lowest in breeds Pietrain and Duroc (1.58 cm) (3).

Orientation of research in the near future: The project is in the second year of its duration. Its completion is planned in 2004. In the subsequent period we will continue with observations of the occurrence of foramen ovale patens according to individual lines of pig breeds commonly used for fattening in our country. We plan to observe the occurrence of this heart defect also in other breeds and lines of pigs that may be used for fattening under our conditions.

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FOLIA VETERINARIA, 48, 1, Supplementum: S27, 2004

INSULIN-LIKE GROWTH FACTORS (IGFS) AND THEIR BINDING PROTEINS IN THE AMNIOTIC FLUID OF ANIMALS

Project VEGA SR, No. 1/0616/03 (formerly 1/8022/01): Duration of the project: from 01/2000 to 12/2002, principal Investigator: Prof. Ing. J. Blahovec, PhD, Department of Chemistry, Biology and Biochemistry of UVM Košice

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Cell growth in mammals is regulated by a growth hormone, insulin-like growth factors (IGFs), specific binding proteins, and cellular receptors. The importance of IGFs and their binding proteins in the regulation and control of normal foetal growth is evident, however, as it is not well understood and sufficiently elucidated it represents an interesting field of current research. Our previous studies have shown, that the amniotic fluid of sheep collected at the 10th week of gestation contained mitogenic-active molecules that can be attributed to IGFs and related molecules (1).

The investigated material was delipidated and purified by size exclusion chromatography and anion exchange chromatography. All chromatographic fractions were lyophilised and tested for mitogenic activity in the system with radioactive thymidine in which mouse fibroblasts were the target cells. Our further studies (Western immunoblot and ligand blot) showed that the higher-molecular-mass, mitogenically active fraction of ewes and goats contained significant amounts of IGF-binding proteins (2). Recently, we have shown that the mitogenic activity of the sheep amniotic fluid is a sum of activities of IGFs and the so-called high molecular form of IGF-II (3).

Orientation of research in the near future: Our studies will be carried out on the amniotic fluid collected from animals in the second and third trimester of gestation. We will utilise our previous experience with purification of amniotic fluid based on chromatographic techniques, Western ligand blot and immunoblot and extend them by investigations of the immunomodulatory properties of individual fractions of peripheral blood lymphocytes. Our investigations will not only broaden our knowledge about the amniotic fluid pattern of IGFs and their binding proteins in different periods of gestation but also bring new information about the effects of these growth factors on immunocompetent cells.

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FOLIA VETERINARIA, 48, 1, Supplementum: S28, 2004

BOVINE PAPILLOMATOSIS

Project VEGA SR, No. 1/0554/03: Duration of the project: from 01/2000 to 12/2002, principal investigator: Prof. F. Lešník, DVM, DSc, Department of Chemistry, Biology and Biochemistry of UVM Košice

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Five new locations of bovine papillomatosis were in Slovakia described: PD DB, VV (a private farm), PD V, RD K, and S s.r.o. The following examinations were carried out in these herds: haematological, pathohistological, metabolic tests and, according to possibility, papillomas were taken to prepare an autovaccine.

On the basis of our monitoring of the occurrence of bovine papillomatosis in Slovakia during the period of our investigations, we could state that with regard to location and the form of papilloma the serotypes BPV1 and BPV2 were involved in the majority of cases. Our conclusion was that without the use of autovaccines papillomatous animals had minimum chance of recovery (4).

In addition to that, we observed that the immunosuppression associated with bovine papillomatosis can also be related to the occurrence of opportune encephalitozoonosis. By the method of indirect immunofluorescence, antibodies to *Encephalitozoon cuniculi* were detected in 39.4 % of papillomatous cows (titre 1:64—1:256) compared to 10 % occurrence in the non-papillomatous cows (titre 0—1:32). The results indicated that *E. cuniculi* may play an important role as an opportune pathogen in various diseases of cattle associated with immunosuppression (1).

We also investigated the functional activity of blood lymphocytes and neutrophils in bulls with persistent skin papillomatosis. A significantly decreased blastogenic response of lymphocytes to T-cell mitogens, revealed during clinical manifestation of papillomatosis, was also investigated. During the regression it was comparable with the activity of control animals. The phagocytic activity of neutrophils was not significantly changed in affected bulls in comparison with the control animals. Similar activity was observed for the number of leukocytes, neutrophils and lymphocytes.

The ratio of CD3+ and IgM+ cells and the functional activity of blood lymphocytes and neutrophils in bulls with persistent skin papillomatosis. During the clinical manifestation of papillomatosis (persisting for more than 1 year) the blastogenic response of lymphocytes to T-cell antigens was decreased significantly in relation to the intensity of stimulated and non-stimulated cells and to the stimulation index. During the regression of skin papillomas also investigated (surgical removal of large papillomas and autovaccination), a gradual increase in the lymphocyte activity was observed. At the end of our observation period (day 139 following the surgical treatment), the values were comparable with those determined in the controls (3).

The CD3+ and IgM+ cell ratio in the blood and phagocytic activity in affected animals were not changed significantly.

All our observations suggested a close interrelationship between etiopathogenesis of bovine papillomatosis and the environment or environmental suppressants (Cd, Pb, As) including mineral metabolism, which is very important with regard to the food chain. However, we recorded bovine papillomatosis also in regions without an emission load but in animals with generally disturbed health (2).

Our studies also involved a potential analogy between human and bovine papillomatogenesis, particularly in women infected with high and low risk human papillomavirus. Only preliminary results were obtained in this area and are subject to more detailed analyses (5).

Orientation of research in the near future: Our future studies will focus on the possibility of detection and induction of apoptosis in animals affected by papillomatosis as well as on testing some substances that have prospects in the treatment of papillomatosis.

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FOLIA VETERINARIA, 48, 1, Supplementum: S29—S30, 2004

STUDY OF THE CHANGES IN THE IMMUNOCOMPETENCE OF DOGS EXPOSED TO VARIOUS INFECTIOUS AND NON-INFECTIOUS FACTORS AND THE POSSIBILITIES OF THE AFFECT OF IMMUNOMODULATORY SUBSTANCES

Project VEGA SR, No. 1/7020/20: Duration of the project: from 01/2000 to 12/2002, principal investigator: Assoc. Prof. J. Mojžišová, DVM, PhD, Department of Infectious Diseases and Parasitology of UVM Košice

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The study evaluated the degree, duration and form of immunosuppression in dogs resulting from various infectious and non-infectious factors. To reach the project goals immunity status was monitored by tests evaluating phagocytosis (chemotaxis, ingestion, metabolic activity) and the functional activity of lymphocytes (blasttransformation test) and by common diagnostic methods for detection of specific antibodies. Monitoring the immunocompetence of dogs affected by various infectious and non-infectious factors was improved by a method of leukocyte immunophenotyping using a flow cytometry. Clinical patients and experimental animals were examined.

The clinical patients, dogs with chronic skin diseases of various etiology, were examined for quantitative proportion and functional activity of neutrophils and lymphocytes. Our examination revealed significant immunosuppression in dogs suffering from atopic skin diseases, dermatomycoses, demodicosis, and pyodermia.

Atopy and recurrent pyodermia was accompanied by significant reduction of functional activity of neutrophils and lymphocytes. Dogs affected by dermatomycosis exhibited altered functional activity of lymphocytes and unchanged or, in some cases, elevated functional activity and proportion of neutrophils (4). Canine demodicosis was associated with immunosuppression related to the form and duration of the respective clinical disease. The immunosuppression related to demodicosis was induced by both serum factors and modified reactivity of lymphocytes. Contrary to local demodicosis the generalised demodicosis is also accompanied by depression in neutrophils. In all dogs with skin disease the immunosuppression complicated the primary disease process, enhanced the risk of secondary bacterial infection, decreased effectiveness of therapy and worsened the prognosis (1). Immunosuppression was confirmed also in animals with recurrent gastrointestinal infections, bronchopneumonia, and glandular cystic hyperplasia complex (3).

Tests were carried out to evaluate the action of an immunostimulatory substance levamisole in clinical patients. It was observed that the per os application of levamisole at a dose of 3.5 $mg.kg^{-1}$ twice a week in combination with a causal acaricide therapy resulted in much earlier and more pronounced adjustment of phagocytic activity of neutrophils and partial recovery of the functional activity of lymphocytes (2).

Alterations of the immune system, resulting from the action of non-infectious factors (stress, surgery), were observed in experimental animals which underwent ovariohysterectomy while anaesthetised by two different techniques (inhalation and injection). The results obtained allowed us to conclude that trauma, pain and effect of anaesthetics during and after the surgery evoked only transient suppression of phagocytosis but a marked alteration of blastogenic response of lymphocytes persisted for as long as one week after the surgery (5).

The experimental animals were investigated for the course and level of specific and non-specific immunity after primovaccination with a live attenuated vaccine against canine distemper and parvovirosis. The non-specific immunological parameters and the level of specific antibodies determined in dogs suffering from giardiosis associated with immunosuppression were compared with those found in dogs with giardiosis treated with an immunostimulatory dose of levamisole.

Both groups of dogs showed alterations in the production of specific antibodies compared with healthy vaccinated dogs. The onset of specific immunity in dogs with giardiosis was delayed by one week in comparison with the dogs treated with levamisole. At the beginning of observation the proportion and functional activity of phagocytes and lymphocytes in the experimental group were lower, however, after the levamisole application an adjustment in these parameters was observed.

Orientation of research in the near future: Our future research will be oriented on the interaction between the infectious

agent and the immune system, elucidation of the mechanism of effect on cellular and humoral immunity, evaluation of the relationship between secondary immunodeficiencies and the occurrence of recurrent infections, the protective action of vaccines and effectiveness of therapy. The research will also focus on molecular-genetic methods for detection of agents inducing viral diseases in carnivores.

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FOLIA VETERINARIA, 48, 1, Supplementum: S31, 2004

MOLECULAR-GENETIC CHARACTERISTICS OF LYSSAVIRUSES ISOLATED IN SLOVAKIA AND IN NEIGHBOURING STATES

Project VEGA SR, No. 1/9010/02: Duration of the project: from 01/2000 to 12/2003, principal investigator: Assoc. Prof. R. Ondrejka, DVM, PhD, Department of Infectious Diseases and Parasitology of UVM Košice

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On the basis of previous ecological and epizootiological studies directed towards obtaining new knowledge about autochtonous populations of the reservoir species of rabies virus as well as the reservoir species of European bat lyssaviruses (EBL) it has been determined that the common fox (*Vulpes vulpes*) is the principal reservoir species in the territory of the Slovak Republic (1). The Racoon dog (*Nyctereus procionides*) is of little importance as a rabies reservoir species in Slovakia (1). Additional knowledge was obtained about autochtonous populations of bats in The Slovak Republic directed mainly towards the crucial reservoir species of European bat lyssaviruses (EBL) – serotine bat (*Eptesicus serotinus*) and pipistrelle bat (*Pipistrellus pipistrellus*) (1, 2). 15 lyssavirus strains were analysed for the purpose of molecular-genetic identification of lyssaviruses isolated from bats and foxes in Slovakia (3—5). All these strains sequenced in our laboratory were very similar to strains isolated in the neighbouring states which suggests an interaction or close relationship of terrestrial epizootic rabies cycles in the central European region (3).

By means of the direct sequencing of the amplification product of the nested reverse transcriptase polymerase chain reaction (nRT-PCR) gene a bat strain classified as a genotype 5 — EBL 1a was isolated. Identification of a lyssavirus EBL 1a, isolated from a bat (*Eptesicus serotinus*), indicates the possibility of the existence of infected bats in Slovakia (4).

Orientation of research in the near future: Our studies will concentrate on obtaining of new knowledge about the biological properties of autochtonous strains of European bat lyssaviruses (antigenic structure, pathogenicity including quantification of the virulence of autochtonous chiroptera and terrestrial mammals strains). Identification and sequencing of isolates obtained from bats and terrestrial mammals by means of reverse transcriptase polymerase chain reaction (RT-PCR) will be provided. Our investigations has been aimed at obtaining epizootiological data about individual species of bats that occur in Slovakia including their age, sex and location, but also a phylogenetic analysis focused on reconstruction of geographic and temporal distribution of EBL in Central Europe.

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FOLIA VETERINARIA, 48, 1, Supplementum: S32, 2004

BVDV CONTROL IN EUROPE

Thematic network of 5th Framework Programme of European Union, No. QLRT-2001-01573): Duration of the project: from 10/2002 to 10/2005, partner 15: Assoc. Prof. Ing. Š. Vilček, DSc, Department of Infectious Diseases and Parasitology of UVM Košice

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The European Commission, Research Directorate-General, has within the 5'th Framework Programme signed a contract supporting the Thematic Network on Bovine Viral Diarrhoea Virus (BVDV) control, in Europe. The first network meeting was held in Copenhagen in December, 2002. The network has since started on its task of summarising the current knowledge on BVDV control and evaluating the future options for its control in Europe.

BVDV is a costly infection for European farmers. Losses are estimated to range from 5 to 20$ per calving in the European cattle population. Scandinavian countries and also a few other regions in Europe have had success with the control of BVDV without the use of vaccines and are aiming towards its eradication. The eradication strategy was found to be cost-beneficial already in the second year of the programme in Norway. After 10 years, Norway has now only 20 BVDV restricted herds left compared with almost 3000 in 1994.

The other principal control measure used in Europe today is vaccination. Further development is necessary for vaccines to be efficient and through the development of diagnostic tools and/or marker vaccines a combination of vaccination and eradication could be possible.

The network has split its work into four working groups with the following goals:

1) to harmonise diagnostic tools and suggest future diagnostic improvements,

2) to identify risks of re-infection for European livestock and suggest control measures,

3) to harmonise vaccines and suggest vaccine and vaccination strategy improvements, and

4) to identify the socio-economic outcomes of the different control measures.

The outcomes of the network will be disseminated through a position paper, seminars, press releases and through the BVDV control web site: www.bvdv-control.com

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FOLIA VETERINARIA, 48, 1, Supplementum: S33—S34, 2004

THE GENETIC VARIABILITY OF PESTIVIRUS GENOME, APPLICATION IN MOLECULAR DIAGNOSIS AND EPIZOOTIOLOGY

Projects VEGA SR, No. 01/6215/99 and 1/9014/02: Duration of the project: from 01/2000 to 12/2002, principal investigator: Assoc. Prof. Ing. Š. Vilček, DSc, Department of Infectious Diseases and Parasitology of UVM Košice

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Altogether 78 strains isolated in 7 European countries in the last four to five years were analysed for the purpose of the study of the evolution of BVDV (bovine viral diarrhoea virus). DNA fragments from a 5'-untranslated region (5'-UTR) and Npro region (encoding viral autoprotease) were prepared by the RT-PCR method. DNA products were sequenced and analysed by computer phylogenetic analysis using a software package Phylip. The analysis included also a portion of nucleotide sequences for BVDV strains obtained from the GenBank. Phylogenetic trees showed that BVDV-1 strains did not form only two up-to-date described groups – BVDV-1a (NADL) and BVDV-1b (Osloss), but at least 11 genetic groups, e.g. BVDV-1a to BVDV-1j and Deer (1). This indicated that the evolution of BVDV-1 has been much more complicated than has ever been suspected. This knowledge is important for the selection of PCR primers for detection of BVDV-1 and also for the selection of strains used for preparation of vaccines.

In general, no significant relationship was observed between the clustering of viruses into phylogenetic groups and their geographic origin. Despite that it was observed that the strains from Austria where five genetic groups have been identified, formed groups similar to those of Mozambique strains. Epizootiological investigations indicated that Mozambique farmers purchased a part of their cattle in Austria. Similar phylogenetic grouping was observed for a strain isolated from imported cattle in Slovakia and the BVDV-1 strains identified in the country from which infected cattle were imported (2).

Besides the BVDV-1 strains, the analysis of pestiviruses allowed us to identify also two BVDV-2 strains originating from France (1). The BVDV-2 isolate was proved for the first time also in Austria and Slovakia (3). These data indicate that the pestivirus BVDV-2, identified for the first time in Canada and the USA in the early nineties, has been spreading gradually also to the central European region.

Modification of the RT-PCR method using pan-pestivirus 324/326 primers selected from 5'-UTR, as well as adapted electrophoretic analysis of PCR products enabled to use this genetic method for analysis of a large number of clinical samples (hundreds to thousands of them) and identify persistently BVDV infected cattle within the BVD/MD eradication programme in Lower Austria (4).

Genetic analysis of cytopathic BVDV-1 strains in the NS2-3 region of the pestivirus genome indicated that the cellular insertion of the cINs type, detected for the first time in BVDV-1 strain NADL, was identified in 8 out of 33 analysed strains. The length of these insertions that were located in the reading frame with the viral nucleotide sequence varied in the range encoding 89—140 amino acids. Two of these analysed strains contained at the 5' or 3'-ends of the cINs insertion another unknown sequence encoding 14—15 amino acids (5). This information is important in understanding the complex mechanisms of development of cytopathic BVDV-1 strains that occur in cattle affected by mucosal disease (MD).

Orientation of research in the near future: Future research will focus on the development of new diagnostic approaches based on DNA *in vitro* amplification techniques for identification of important viral pathogens of farm animals. We will also focus our research on genetic typing of pestiviruses and other viruses, their evolution, and the practical use of knowledge obtained in the field of molecular epizootiology of animal viral infections. A part of our research capacity will be directed towards the study of variability in the E2 encoding region of pestivirus genome looking for correlation between data obtained by antigenic and genetic analysis.

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FOLIA VETERINARIA, 48, 1, Supplementum: S35, 2004

SYSTEMIC AND LOCAL IMMUNITY OF POULTRY AFTER *Eimeria* spp. INFECTION WITH DIFFERENT PATHOGENICITY

Project VEGA SR, No. 1/7018/20: Duration of the project from 01/2000 to 12/2000, principal investigator:
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The most suitable monoclonal antibodies to chicken immunocompetent cells were tested and selected for flow cytometry measurement in the peripheral blood. The infection of Leghorn chicks with specific pheasant coccidia *Eimeria colchici* resulted in the dispersed, impotent invasion of the caecum and small intestine of these birds. The development of the schizonts in the intestinal mucosa was inhibited and the schizont were significantly smaller (5.7 × 3.8 µm) then those in natural hosts (18.7 × 15.1 µm). A general increase in the number of peripheral white blood cells and particularly that in CD4+ cells indicate that these cells participated in the defence process during the invasion and development of coccidia in a non-specific host (1).

In comparison with that, experimental *Eimeria colchici* infection in specific hosts resulted in leukocytosis, improved phagocytosis of heterophiles, and higher proliferative lymphocyte response to this parasitic agent, observed from the beginning of the infection. The changes in peripheral blood heterophiles and lymphocytes suggested an important role of the cell-mediated immune response in this disease (2).

Application of *Eimeria colchici* to non-specific host – chickens, induced systemic and local immune response, particularly the cells-mediated immune responsiveness. The number of eosinophilic granulocytes and CD3 positive lymphocytes in the *duodenum* and *caecum* increased during the infection period (4). Evaluated number of CD3+, CD4+, CD8+, Bu1b+ cells and ratio of CD4/CD8 in both, the peripheral blood and spleen reflected a statistically significant increase of CD4+ cells at 60 hours post infection in the peripheral blood. *Eimeria colchici* had immunogenic effect to non-specific host, chickens (5).

The immunocytochemical study of the local immune response after long-term application of aflatoxin B1 showed a decrease in CD3 T-cell in the intestinal mucosa. Flow cytometry determined a decrease in peripheral white blood cells. Evidently, application of similar immunosuppressive substances may influence the development of *Eimeria colchici* schizonts (3).

Orientation of research in the near future:

– Preparation of pheasant monoclonal antibodies could help to determine systemic and local immune response and immune mechanisms induced by *Eimeria* infection in these birds.

– The immunogenic effect of *Eimeria colchici* on chickens may be used advantageously in future studies of parasitic antigens and may help to prepare effective coccidial vaccines.

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FOLIA VETERINARIA, 48, 1, Supplementum: S36, 2004

THE NEURO-IMMUNO-HUMORAL REGULATION OF APOPTOSIS IN B- AND T-LYMPHOCYTES. THE TOTAL ANTIOXIDATIVE STATUS IN PATHOGENESIS OF IMMUNOSUPPRESSION AND SOME SELECTED DISEASES

Project VEGA SR, No. 1/0581/03: Duration of the project: from 01/2003 to 12/2005, principal investigator: Prof. I. Škardová, DVM, PhD, I Internal Clinic of UVM Košice

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The basic aim of the project is the study of mechanisms of neuro-immuno-humoral regulation of apoptosis in B-and T-lymphocytes in relation to the total antioxidative status (TAS) in poultry. Disorders of these mechanisms play an important role in the pathogenesis of acquired immunodeficient states with serious economic consequences. These are the mechanisms through which various factors (pathogens, stressors, nutrient factors, radiation, etc.) participate individually or in combination in inducing various health disorders. Their role in the pathogenesis of the immunosuppression of the states mentioned have not yet been uniformly explained in avian medicine and their final accurate detection requires new target methods (1, 2, 3) in the project.

The increased metabolic activity of chickens on intensive farms places high demands on the quality of environmental conditions. Numerous factors can affect the mechanisms important for poultry production positively or negatively. Target studies specialising on mechanisms that optimise the growth, production of muscles and similar traits in poultry were conducted on a worldwide scale. Our previous studies between 2000 and 2003 conducted under experimental and practical conditions pointed to various mechanisms, which may participate in the development of immunodeficient states and are limited by the health and immune status and quality of the feeding regimen. The results showed that defects arise as a consequence of biochemical, structural, and cellular alterations due to damage to DNA and proteins and cause disorders in energy metabolism (4, 5).

Orientation of research in the near future: Research will be oriented on development of methods for determination of apoptosis in B- and T-lymphocytes, detection of ROS (reactive oxygen species) and neuro-immuno-humoral structures.

The future research will focus on detection of parameters in superoxide dismutase – SOD, glutathione peroxidase – GPX, catalase – KAT, glutathione reductase – GR, reduced glutathione – GSH, oxidised glutathione – GSSG and GSSG/GSH ratio in erythrocytes in poultry.

Part of the research programme will be devoted to the evaluation of some products of lipide peroxidation, vitamin E and C, and uric acid in blood plasma in relation to apoptosis.

Attention will be paid to the development of methods for the evaluation of apoptosis morphometrically and with apostain and to selected histoenzymatic methods.

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FOLIA VETERINARIA, 48, 1, Supplementum: S37—S38, 2004

OPTIMISATION OF SANITATION PROCEDURES IN AGRICULTURAL PRODUCTION WITH THE VIEW OF DECREASING THE RISK OF CONTAMINATION OF THE ENVIRONMENT AND DANGER TO HEALTH

Project VEGA SR, No. 1/7017/20: Duration of the project: from 01/2000 to 12/2002, principal investigator: Prof. M. Ondrašovič, DVM, PhD, Department of the Environment of UVM Košice

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Taking into account the problems related to disinfection, attention was paid to sanitation of animal manure infected with disease agents. Especially topical is the disinfection of manure of animals affected by paratuberculosis. The agent is one of naturally occurring micro-organisms, highly resistant to both external environmental conditions and chemical disinfectants. To devitalise this agent in animal manure Persteril (33—36 % peracetic acid) in the concentration 0.3 % (active ingredient) was applied to 25 cm high layer of manure at a dose of 5 l.m^{2}. After one hour exposure the manure was subjected to composting. The temperature development in the manure treated with Persteril and in the control was recorded with probes introduced into the substrates. The maximum temperature recorded after twenty four hours the control compost and 57 °C in the treated manure. By day 6 the temperatures in both substrates had stabilised at 53 °C. By day 26, the day of termination of the composting process, no viable *E. coli* were found in the piles. Total numbers of micro-organisms decreased by two orders of magnitude in the non-disinfected manure and by three orders in the disinfected one. Of the chemical parameters determined at the end of composting, the biggest differences were observed in the CODCr content that reached 103.6 g.kg^{-1} in the control manure and 53.2 g.kg^{-1} in the disinfected one (1).

The investigations included also evaluation of the effect of various disinfectants on *Ascaris suum* eggs. The highest devitalisation effectiveness was observed with ammonium hydroxide and reached 79.7 % when using the chemical in 0.5 % concentration and 94 % after application of 10 % ammonium hydroxide. The devitalisation effectiveness of other disinfectants investigated in this experiment (chlorine lime, Dikonit and Savo) was lower than 50 % (2).

Additional experiments focused on the possibility of application of Slovak natural zeolite clinoptilolite in the process of aerobic biological treatment of pig excrements and sanitation and further use of the separated solid fraction of pig slurry. The results obtained indicated a positive influence of powder zeolite on bioflocculation and sedimentation of the activated sludge. Investigation of the decomposition processes in the stored pig slurry solids before its agricultural use, amended with various doses of zeolite, was oriented on chemical and microbiological aspects important from the point of view of environmental protection and devitalisation of potentially present agents of diseases (3). Investigations in the respective substrates showed that addition of 1 % and 2 % zeolite by weight to the pig slurry solids affected absorption and gradual release of ammonia and subsequent nitrification processes in relation to storage conditions (4). The viability of coliform and faecal coliform bacteria during the storage of the solid fraction indicated an influence of zeolite depending on its dose and the period of storage contributing to the devitalisation of indicator micro-organisms.

Orientation of research in the near future: Our investigations of disinfectant action will focus on selection or combination of such chemical compounds that exhibit a wide range of effectiveness with regard to unwanted microflora including agents of parasitic diseases. Attention will also be paid to their undesirable effects on the environment and to such methods and procedures that can minimise these potential negative effects. With regard to the use of zeolites, we will continue to focus on their application in the final treatment of waste-waters and composting of organic materials where these materials appear to have prospects.

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FOLIA VETERINARIA, 48, 1, Suplementum: S39, 2004

DIGESTIVE DISORDERS OF WEANED PIGLETS WITH FOCUS ON THE DIARRHOEIC SYNDROME

Project VEGA SR, No.1/7014/20: Duration of the project: from 01/2000 to 12/2002, principal investigator: M. Húska, DVM, PhD, II Internal Clinic of UVM Košice

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The studies conducted within the project allowed us to observe, describe and define the dominant functional and structural changes in the gastrointestinal tract (GIT) induced by the presence of enteropathogens causing digestive disorders, particularly after the weaning of piglets, concentrating on the diarrhoeic syndrome.

We used new diagnostic-laboratory methods and target clinical examination directed towards the observation of qualitative changes in the intestines after supplementing the feed with selected probiotic lactobacilli strains (1).

The study summarises the results of the investigation of the digestive tract in relation to external influences, such as the ecology of the environment, feed components and parasites, and to internal factors including GIT microflora and the development of immunity that interact with each other and provide the overall picture of animal health (2).

Experiments were carried out to test the influence of probiotic strains *Lactobacillus plantarum* LB5, *Lactobacillus reuteri, Lactobacillus casei,* and *Bacillus licheniformis,* using strain *E. coli* K88 as enteropathogen. Their effect on the incidence of the diarrhoeic syndrome in piglets was investigated in all experiments with an emphasis on clinical examination and evaluation of haematologic, immunologic, proteinaceous, energetic and mineral profiles and the effect of pH on the intestinal mucosa and weight gain of piglets. Monitoring of metabolic, invasive and infectious diseases was conducted under field conditions.

The study summarises the effects of *Lact. casei* subsp. *pseudoplantarum* No. 294 on the intensity of growth of pigs and the influence of metabolic markers in pigs after oral application. We observed the health status of pigs in relation to the development of the diarrhoeic syndrome focusing on selected parameters of the immunological profile and health and stimulation of growth through decreasing pH of GIT, which appeared beneficial.

Probiotics as natural bioregulators help to maintain the balance and optimise of intestinal microflora of GIT and prevent its colonisation with pathogenic bacteria. The experiment showed a positive effect on the immune status of animals which was reflected in the growth and weight gains of piglets and decrease in pH values in GIT of animals by the application of *Lact. planarum* LB5. Light and electron microscopy observations confirmed the adherence of the autochtonous strain *Lact. planarum* LB5 to the small intestine mucosa, which prevented the development of diarrhoeic syndrome following the application of enteropathogenic *E. coli* K88 to these animals (3). This micro-organism did not affect the haematological profile of pigs (number of Er and Lc, concentration of Hb, Hk and MCV). A statistically significant effect on the activation of oxidative metabolism of phagocytes (INT test) and phagocytic activity (PA) was observed. Hypoproteinemia and hypoalbuminemia was observed throughout the experiment while the serum glucose was not affected. Probiotics affected positively the health status of piglets which was reflected in stimulation of their growth and higher weight gains.

The probiotic strain *Lact. plantarum* LB5 was used to test the active protection of GIT against enteropathogenic *E. coli* 08:K88 imparted by the probiotic. A positive effect on the immune system and thus also on the health of these animals was observed.

Orientation of research in the near future: The development of desirable and stable microflora of GIT in neonatals appears to be a process essential to the health and productivity of animals and important also to humans. The results obtained during our work on the project can have immediate application in practice in coping with serious health and economical problems related to frequent diarrhoeal, respiratory and metabolic diseases in weaned piglets. We will prolong our research in this direction.

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FOLIA VETERINARIA, 48, 1, Supplementum: S40, 2004

ANALYSIS OF THE PRESENT LEGAL SITUATION CONCERNING TRANSGENIC ANIMALS AND PREPARATION OF A LEGAL STANDARD ABOUT BREEDING AND KEEPING TRANSGENIC ANIMALS

Project VEGA SR, No. 1/8023/01: Duration of the project: from 01/2001 to 12/2002, principal investigator: Prof. A. Bugarský, DVM, LLD, PhD, Department of Food Hygiene and Technology of UVM, Košice

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The objectives of the project were split into two stages. The aim of the first stage was to analyse all the effective legal regulations that apply to the breeding and keeping of experimental animals and the legal regulations of the EU that stipulate the management of transgenic animals. The aim of the second stage was to prepare a paragraph version of a legal proposal concerning the management of transgenic animals.

The principal output of the project team's efforts should be a version in paragraph of a proposal of legal regulation controlling the field of transgenic animals. In the first half of 2002, the National Council of The Slovak Republic adopted Act. No. 151/2002 of the Code about the use of genetic technologies and genetically modified organisms (GMO). Subsequently, the Ministry of the Environment of The Slovak Republic issued Regulation No. 252/2002 of the Code intended for the implementation of Act No. 151/2002 of the Code (3, 5).

Both legal standards regulate the field of genetically modified organisms (including transgenic animals) in a way which basically agrees with our original conception. Our objections are directed at certain provisions of Act No. 151/2002 as well as Regulation No. 252/2002 of the Code. With regard to the extent of space available for the presentation of results obtained during the work on the project we do not present them as a complex. The systematic presentation of results of the analysis of both legal standards mentioned above and of our proposals was published in the monograph prepared by the authors' collective (1).

The principal differences between the effective legal standards and our conception of the legal handling of the problems related to genetic modifications consist of a different view of the classification of GMO-handling personnel into risk classes and about the scope and operation of state administration bodies active in the field of GMO (2, 4). Objections to Regulation 252/2002 of the Code relate to to the method of specification of the eligibility for the performance of work related to the GMO, method and periodicity of the specialised education of personnel working with the GMO and questions related to assigning workplaces into respective risk classes.

Direction of research in the near future: With regard to the expected accession of SR to the EU and the resulting surge of investment envisaged also in the field of modern genetic technologies we assume more extensive use of techniques intended for preparation of GMO. With regard to this we plan to focus our attention on the method of registration of GMO, particularly GM animals, control of the movement of GMO products in relation to existing legal standards. Our research will be carried out also in the field, particularly through distributing questionnaires to subjects concerned in different way with the problems related to GMO.

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FOLIA VETERINARIA, 48, 1, Supplementum: S41, 2004

THE RELATIONSHIP BETWEEN THE POLYMORPHISM OF BACTERIAL AND EUKARYOTIC GENES AND THE DISEASES

Projects VEGA SR, No. 1/6232/99, 1/8021/01, 1/0433/03: Duration of the projects: from 01/2000 to 12/2005; project: AV/802/2002-05: Duration of the project: from 01/2002 to 12/2005, principal investigators: Prof. I. Mikula, DVM, DSc, Assoc. Prof. Ľ. Tkáčiková, DVM, PhD, Laboratory of Biomedicinal Microbiology and Immunology of UVM Košice and Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava

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1. The proportion of individual risk PrP genotypes in sheep of the Slovak Valachian breed was determined in the study of the polymorphism of genes of sheep participating in the immunity to scrapie by means of genotyping of the *PrP* gene (focusing on comparison of codons 136, 154 and 171) (3). This information is needed for the positive selection of sheep with a low susceptibility to this disease (6).

2. The study of the virulence genes polymorphism of *Streptococcus agalactiae* strains isolated from human and animals resulted in the following:

– The analysis of the genetic virulence determinants of *S. agalactiae* strains of human and animal origin demonstrated the differences between these strains (1, 8, 10).

– New approaches for the analysis of genetic polymorphism for differentiation of *S. agalactiae* strains of human and bovine origin were developed (PCR, ribotyping, PFGE) (4, 7).

– A comparative analysis of *S. agalactiae* strains of human and bovine origin based on the presence of different insertions sequences was performed (PCR, Southern hybridization) (5, 9);

3. The comparative study of *Listeria monocytogenes* strains isolated from different sources (milk and milk products) by pulsed field gel electrophoresis and ribotyping was performed in order to analyse their genomic DNA. This study revealed the epidemiological relationship between some of the *L. monocytogenes isolates* (2).

Orientation of research in the near future: Future research will be directed towards the following:

1. The study of polymorphism of the*PrP* gene in sheep of other breeds reared in Slovakia (Tsigaja, Merino and East-Friesian).

2. The study of polymorphism of genes of the MHC class II in cattle and sheep in relation to topical bacterial diseases.

3. The study of immune response to *S. agalactiae* antigens.

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FOLIA VETERINARIA, 48, 1, Supplementum: S42, 2004

NEW TRENDS IN THE IMMUNOPROPHYLAXIS OF FARM ANIMALS

Project VEGA SR, No. 1/8025/01: Duration of the project: from 01/2001 to 12/2003, principal investigator: Assoc. Prof. E. Holoda, RND, PhD, Department of Food Hygiene and Technology of UVM in Košice, Centre for Analysis of DNA of UVM in Košice

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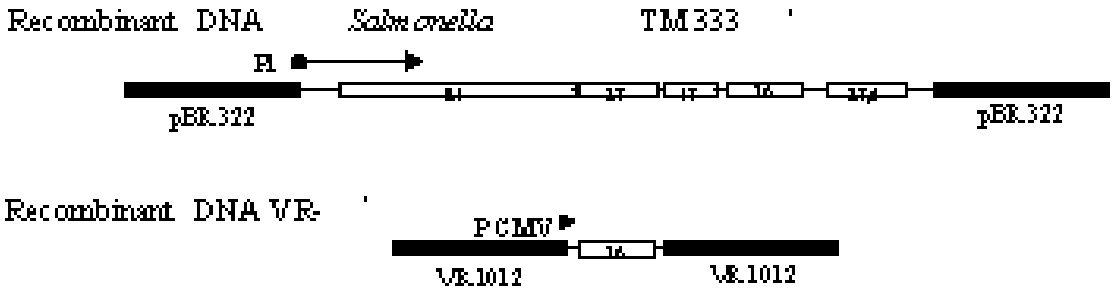
The aim of the project is to investigate the qualitative and quantitative parameters of immune response after application of vaccine with recombinant DNA and vaccines that code for K88 antigen in dependence on the host bacterial strain, the method of application and the application dose.

Recombinant DNA containing gene encoding the K88ab antigen in the vector VR1012 (VICAL INC., San Diego, USA) was prepared. The recombinant VR1012-K88 DNA intended for immunisation was prepared as follows: *E. coli* M1 PCR gene coding for K88ab antigen was amplified. The K88ab gene treated with respective enzymes was ligated with the vector VR 1012 and transformed by *E. coli* DH5α F^- (1). Rec DNA was analysed by restriction endonucleases. DNA intended for immunisation was isolated from the obtained bacterial colony of presumed composition (marked VR 1012-K88) (2, 3).

The highest titre of antibodies after application of DNA vaccines was recorded in the case of i. m. administration (1: 320). This is the exact method of its frequent administration which appears successful and is relatively undemanding. Antibody titres were detected also in progeny. No satisfactory humoral response was obtained in mice that were vaccinated *per os* (1: 40). Similarly, intradermally administration induced titres (1: 160) of anti-K88ab antibodies that were lower than those after i.m. administration (4).

Orientation of research in the near future: To observe the affect of application of DNA-K88 vaccines to pigs and preparation of other relevant DNA vaccines.

At the same time, using DNA diagnostic methods (analysis of genomic and plasmid DNA, PCR), to observe the occurrence of bacterial pathogens in farm animal herds. Further



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FOLIA VETERINARIA, 48, 1, Supplementum: S43, 2004

MICROSPORIDIOSES AS OPPORTUNE INFECTIONS AND THE USE OF PRIMERS FOR IDENTIFICATION AND DIAGNOSTICS OF MICROSPORIDIA BY THE PCR METHOD

Pr*ojects VEGA SR, No. 1/7022/20 and 1/0580/03: Duration of the projects: from 01/2000 to 12/2002, principal investigator: P. Bálent, DVM, PhD, Department of Chemistry, Biology and Biochemistry of UVM Košice*

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Microsporidia are obligate, intracellular and sporulating parasites capable of infecting both vertebrates and invertebrates. The infection is commonly manifested by diarrhoea, *sinusitis, keratoconjunctivitis, nephritis, hepatitis* or reproductive dysfunction. The seriousness of disease may range from asymptomatic infections to lethal respiratory or renal failures.

– Our institute has analysed and identified a genome from the *Encephalitozoon cuniculi* used in our study (1). By means of PCR and specific sets of primers for *Encephalitozoon cuniculi (5´ATGAGAAGTGATGTGTGTGCG 3´: 5´TGCCATGCACTCACAGGCATC 3´) a pair of primers was used to amplify a 549-bp product of a small subunit of ribosomal RNA (ssrRNA) from E. cuniculi spores of cell culture cultivated and used for antigen production in our laboratory. Both pairs of primers used (ECUNF 107N4: ECUNR 107N5, Generi Biotech, CzR) were species specific. The primers were amplified at the positions 344—364 and with the reverse primer at the position 872—892. These nucleotide positions coincided with the sequence of a small subunit of rRNA registered in GenBank under registration No. L17072. The PCR amplification was performed using a standardised kit QIAquick PCR Purification (QIAGEN, Valencia, USA).*

– *The immune profile was evaluated under experimental in vivo* conditions on the basis of the influence of *E. cuniculi* on the immune system of susceptible animals. Observation of the dynamics of antibody immune response in intraperitoneally infected rabbits showed that antibodies specific to *E. cuniculi* were detectable already in the early phase of the infection. Similarly, the phagocytic index, i.e. ingestion ability of potential phagocytes and the blastogenic response of T-lymphocytes to mitogen concanavalin A and phytohaemagglutinin, was decreased significantly during the early phase of the infection when the absolute values of CD4+ T-lymphocytes, CD8+ T-lymphocytes and CD19+ B-lymphocytes were also decreased. The CD4/CD8 T-lymphocyte ratio was lower in all experimental groups in comparison with the control (3, 4).

– When evaluating the changes in serum levels of urea and creatinine in experimentally infected rabbits we observed a significant decrease in the concentration of creatinine and an increase in serum urea in the infected animals. The influence of an azol derivative, albendazol, on the development and course of experimental encephalitozoonosis was evaluated from the point of view of its potential use in the therapy of the disease that has not yet been successful under *in vivo* conditions. Administration of albendazol after previous experimental infection with *E. cuniculi* decreased the antibody immune response in infected rabbits which indicated a relevant effect of albendazol on the course of microsporidiosis (2).

Orientation of research in the near future: The polymerase chain approach (PCR) will be used to develop a simple but effective technique that will allow us to amplify the nucleotide sequences of DNA fragments of microsporidia by means of a polymerase chain reaction with a small subunit ribosomal RNA (rRNA). The use of microsporidial rRNA primers may contribute considerably to the study of epidemiology or to investigation of the zoonotic potential of microsporidia. The PCR process can directly detect the infectious agent which brings the advantage of early diagnosis associated with high precision and specificity. Introduction of this superior, rapid, and simple diagnostic method creates conditions for prompt prophylactic and therapeutic interventions.

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FOLIA VETERINARIA, 48, 1, Supplementum: S44, 2004

THE INFLUENCE OF CHANGED ECOLOGICAL CONDITIONS ON THE HEALTH AND PRODUCTION OF ANIMALS, FISH AND BEES

Project VEGA SR, No. 1/7041/20: Duration of the project: from 01/2000 to 12/2002, principal investigator: Prof. V. Letková, DVM, PhD, Department of Infectious Diseases and Parasitology of UVM Košice

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Selection of most pathogenic *Eimeria* species invading pheasants and partridges kept on farms allowed us, through experimental infection, to obtain more accurate knowledge about the endogenous development of *Eimeria colchici* and *E. duodenalis* in pheasants (*Phasianus colchicus*) and *E. procera* in partridges (*Perdix perdix*). According to the international standard methods (COST 89/820), these highly pathogenic species of *Eimeria* have been kept under laboratory conditions (1). Our observations of the immune response of lymphocytes in pheasant chicks infected with *E. colchici* provided important information about metabolic activity of phagocytes and blasto-transformation of lymphocytes (2). Cellular immunity was determined during the infection with *Eimeria colchici* in specific (*Phasianus colchicus*) and non-specific hosts (*Gallus domesticus*). Mutual interaction of carbohydrates and lectins on the surface of schizonts and gamonts of *Eimeria* spp. and intestinal epithelium of pheasants after experimental infection was determined by cytoagglutination with saccharides and glucoconjugates (3, 4).

The health, production and quality of fish muscles, depend on water quality. Research is directed towards observation of residues PCB and heavy metals and the occurrence of ekto- and endoparasites of fish as well (5).

Orientation of research in the near future: Prevention of coccidiosis on pheasant and partridge farms, development of immunisation strategies based on attenuated strains of the most pathogenic *Eimeria* species. The use of vaccination, the latest trend in protection of farms keeping feather game and in rearing healthy pheasants and partridges, also for protection of the environment loaded with air pollutants and other contaminants. To stimulate the protective anti-coccidial immunity by administration of live attenuated pathogens.

Detection and potential elimination of PCB and heavy metals in the fish muscles obtained from selected localities. Observation of influence of pollutants on the health of fish.

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FOLIA VETERINARIA, 48, 1, Supplementum: S45, 2004

THE CHARACTERISTICS OF PARAZITOZOONOTIC AGENTS, EPIZOOTIOLOGY AND THE DIAGNOSIS OF DISEASES THEY INDUCE USING MOLECULAR AND IMMUNOLOGICAL APPROACHES

Project VEGA SR, No. 2/1150/21: Duration of the project from 01/2001 to 12/2003, principal investigator SAS SR: Prof. P. Dubinský, DVM, DSc, Parasitological Institute; principal investigator ME SR: V. Revajová, DVM, PhD., Department of Pathological Anatomy, Pathological Physiology and Genetics of UVM Košice

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The project deals with the problems arising from helminthozoonotic disease – larval toxocarosis in non-specific hosts. The migratory behaviour and histological and immunological responses of the parasite were studied in two different paratenic hosts, mouse and sheep, infected with infective *Toxocara canis* eggs.

It was found that the migratory pathways of *T. canis* larvae and histological responses of both animal species were similar except for some minor differences. Single and repeated infections did not modify the classical histological response in the affected organs. Liver granulomas and severe *T. canis*-induced pulmonary inflammation were the main lesions. This observation is important for public health, as it has been assumed that a single high infective dose may result in severe pathological outcomes. Therefore a polluted neighbourhood presents a potential risk to human health.

Histological changes in the *ileum* and the immunocytochemical detection of the *T. canis* antigen in this part of the small intestine indicated it to be the major predilection site of intestinal infection by paratenic hosts.

The brain is considered to be a suitable organ for harbouring *T. canis* larvae. Abundant larvae were detected in cerebral tissue in mice without any immune cell reactions (1). The neurotrophic phase and histological changes could not be detected in sheep after administration of a single infective dose (2). The duration of the infection and the size of inoculum might be considered important factors.

The formation of eosinophilic granulomas and activation of macrophages are known to be CD4+-dependent reactions in mice. The formation of granulomas in sheep was accompanied by the demonstration of CD4+ cells detected by immunocytochemistry methods. It can be assumed that the reaction follows the same pattern in mice. The detection of CD8+ cells in sheep granulomas indicated that they are important to the process of the destruction of parasitic larvae (2).

Larval toxocarosis is often overlooked in vertebrates. Histological observations in lambs may throw some light on the pathology of larval toxocarosis in this animal species and the introduction of sheep as an experimental host model widely usable for the study of this disease in the future. Demonstration of localisation of *T. canis* antigens in the small intestine, liver, and lungs supports the assumption of migratory behaviour of *T. canis* larvae in these animals (1, 2, 3).

The flow cytometry detection of T- and B-cell subpopulations demonstrated that the application of *T. canis* eggs initiated changes in the systemic immune response. The administration of multiple infective doses to mice elicited various changes in their immune response while single infective doses administered to sheep induced more uniform immune responses. Significant changes in neutrophiles and eosinophils appeared to be connected with phagocytic activity and the killing of parasites. Significant modulation of CD2, CD4, CD8 T-cells, IgM bearing cells, and macrophages seems to be associated with antibody production and destruction of parasites (4, 5).

Orientation of research in the near future: It appears in general that helminth antigens stimulate preferentially Th2 responses while Th1 responses may be of little protective benefit. Investigations will be carried out to determine the secreted interleukins in the sense of interactions between the immune effector cells. This will help to determine precisely the type of T-helper cell response in this animal species, which is induced by larval toxocarosis.

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FOLIA VETERINARIA, 48, 1, Supplementum: S46—S47, 2004

THE TOTAL AND LOCAL IMMUNITY OF POULTRY INFECTED WITH *Salmonella* spp.

Project VEGA SR, No. 1/8117/01: Duration of the project 01/2001 to 12/2003, principal investigator:
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One day old chickens were infected with two different doses (low – 2×10^2 CFU and high – 2×10^8 CFU) of pathogen *Salmonella enteritidis* PT4 and the following changes were observed:

– non-absorbed yolk-sac, diphtheroid *typhlitis* and *enteritis, pericarditis* and *peritonitis,*

– singular lympho-epitheloid granulomas in blind sacs (1).

After administration of the low bacterial dose (2×10^2 CFU) we failed to observe any clinical symptoms despite the fact that bacteriological examination confirmed the presence of this pathogen in the body. Differences in colonisation of organs within one group were observed. The low infective dose of salmonellae produced a total picture similar to that in the flocks infected spontaneously.

Salmonellosis of poultry induced by *S. enteritidis* is difficult to discern clinically and can appear asymptomatic. Common bacteriological methods used on poultry farms are frequently unable to prove the agent particularly in the case of different colonisation of organs in individual birds and the presence of lower numbers of bacteria remaining below the detection limit. The yolk-sac and granulomas may serve as reservoirs of bacteria. *Salmonella enteritidis* is dangerous because of its ability to persist in hosts without causing any disease. It can then easily enter the food chain and cause disease in humans (2).

Electron-microscopic studies revealed that the infection of one day old chickens with *Salmonella enteritidis* PT4 caused an accumulation of heterophylic granulocytes in blind sacs. Morphological changes observed in the chickens indicated degranulation which could result from elimination of antimicrobial peptides. Intracellular changes in cells of the monocytic-macrophage system (oedemas, condensation of chromatin, rupture of nuclear membrane, destruction of cytoplasmic organelles, formation of intracellular inclusions and the presence of bacteria in various stages of destruction) not accompanied by damage to their cytoplasmic membrane did not suggest the death of these cells resulting from experimental infection with *Salmonella enteritidis* PT4.

In the later stages of infection the cytoplasmic membrane is likely to rupture and the content of cells escapes to their surroundings. In this way the cells of the monocytic-macrophage system become the source of the spread of the infectious process to other organs and the process becomes generalised (3).

Flow cytometry study of the immunological profile of chickens experimentally infected with *Salmonela enteritidis* PT4 showed the following:

– increased expression of BU1+ β-cells in all experimental chicks,

– significant increase in CD4+ T-lymphocytes in blind sacs and the spleen from the beginning of the experiment as well as increased expression of CD3+, CD8+ starting from day 7 of the experiment. Increased expression of all subpopulations was observed till the end of the experiment in both groups of chickens.

Time differences in the expression of subpopulations of T-lymphocytes between the two experimental groups were detected. The low dose of bacteria caused a significant increase in the number of lymphocytes up to day 10 following the infection while the high dose induced changes that occurred earlier, were more intensive and lasted longer. The dynamics of changes in the number of lymphocytes indicated that CD4+ T-lymphocytes had a primary effect while CD8+ T-lymphocytes showed secondary action reflected in the development of the immune response to *Salmonella enteritidis* PT4 (4).

Orientation of research in the near future:

1. To apply *Enterococcus* spp. to broiler chicks as a probiotic and to monitor the mechanism, intensity, and the extent of adhesion of bacteria to the intestinal mucosa. To infect subsequently the chicks with pathogenic *Salmonella enteritidis* PT4 in order to observe the bactericidal effects of *Enterococcus* spp. and their ability to inhibit intestinal colonisation with salmonellae.

2. To induce heterophilia in chicks by administration of the parasitic antigen. To infect the chicks experimentally with *Salmonella enteritidis* PT4 and to observe the effectiveness of the bactericidal response (phagocytosis and release of anti-

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FOLIA VETERINARIA, 48, 1, Supplementum: S48, 2004

THE DIVERSITY OF THE AGENTS OF SELECTED TICK-TRANSMITTED PARASITOZOONOSES IN CENTRAL EUROPE

Project VEGA, SR No. 2/3213/23: Duration of the project: from 01/2003 to 12/2005, principal investigator: B. Peťko, DVM, PhD, Parasitological Institute of SAS; substitute investigator: Prof. I. Škardová, DVM, PhD, I Internal Clinic of UVM Košice

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The project investigates the laws of the formation of the biological properties of agents of selected tick-transmitted diseases in the process of evolution of the parasite-host relationship in geographically and geologically diverse regions of Central Europe with stress on the Carpathian system of East Slovakia and Poland and the Czech massif (1).

It is directed towards the genetic and immunologic diversity of agents of serious parasitozoonoses (*Lyme borreliosis, ehrlichiosis, babesiosis* (2, 4) and selected endocrinopathy (3).

It focuses on the occurrence, spread and dominance of epidemiologically and epizootiologically important disease agents in domestic and farm animals and in the human population in comparison with their occurrence in vectors and reservoirs in natural foci (5).

The project presumes the discovery of new genotypes and ecotypes of agents, their reservoirs and carriers, elucidation of additional ways of spreading of infections to humans and animals.

The results will extend the basic knowledge of the diversity of tick-transmitted diseases under the original and changing ecological and climatic conditions of Central Europe.

Orientation of research in the near future: The partners in the project VEGA, the Parasitological Institute of SAS, Košice (project leader), Institute of Zoology of SAS, Bratislava, Epidemiology of LF UPJŠ, Košice and the I Internal Clinic of University of Veterinary Medicine, Košice will continue in this project VEGA collaboration in the future with two bilateral projects of the Polish Academy of Sciences and the Academy of Sciences of the Czech Republic registered in Academic International Agreement (MAD) and another project based on intergovernmental agreement between the Slovak and Czech Republics about (MVTS, 2002—2003). The project receives only partial financing by VEGA (common laboratory operation) while the MVTS and MAD projects permit collection of material in the partner countries (Poland, Bohemia). As the Carpathian system also reaches Hungary, additional collaboration appears desirable. New isolates of disease agents from various organisms and geographical regions will serve as a basis for additional studies of their biological properties and preparation of antigens for specific serologic diagnosis in veterinary and medical practice.

Further research during years 2003—2005 is focusing on the development of methods investigating the laws of formation of biological properties by means of selected, tick-transmitted diseases under the described conditions. Target studies will be conducted concerning lyme borreliosis as a serious factor in public health from the aspect of continuous monitoring, education of the public, prevention, early diagnosis and therapy, and cooperation of specialists from multidisciplinary teams and education centres.

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FOLIA VETERINARIA, 48, 1, Supplementum: S49—S50, 2004

THE DEVELOPMENT OF LIPOID ADJUVANT AND OF RATIONAL METHODS FOR RABIES INJECTION VACCINE EFFECTIVENESS QUANTIFICATION *in vivo*

Project VEGA SR, No. 1/7059/20: Duration of the project: from 01/2000 to 12/2002, principal investigator: Assoc. Prof. Z. Beníšek, DVM, PhD, Department of Infectious Diseases and Parasitology of UVM Košice

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We developed a technological procedure for the preparation of a metabolisable lipoid adjuvant of an oil-in-water type based on a natural areactogenic oil-linear hydrocarbon cholesterol precursor-squalene. Additional components of the emulsion included a mixture of detergents (emulsifiers), namely poloxamer 105 (manufacturer ICI, England) and Abil-Care (Merck, Germany). Poloxamer 105 is a block copolymer with emulsifying and adjuvant properties. It has excellent properties with regard to the mechanism of a combined effect in the preparation of combined adjuvants of the oil-in-water type (HLB = 18.5).

As the second emulsifying component polysiloxane polymer dimethicone copolyol, commercial name Abil-Care (HLB = 10) was used, which produces stable emulsions in the pH range 5.5—9.0 even at laboratory temperature. The emulsification temperatures 35—37 °C permit the preparation of liquid adjuvant vaccines without alteration of the immunogenic activity of the respective vaccination strain and ensure, at the same time, a high stability in emulsions. This technological procedure was used in our laboratory to prepare an adjuvant veterinary rabies vaccine. The effectiveness and safety of this adjuvant rabies vaccine was tested in model experiments on laboratory animals, which indicated the safety of the experimental adjuvant.

The following methods were developed in the process of the improvement of a methodical procedure for quantification of injection rabies vaccines immunogenic activity:

a) A rational method for quantification of rabies vaccines effectiveness based on an *in vitro* method which, on principle, determines the antigenic value of vaccines in the process of their production (semi-product) as well as the final product including adjuvant rabies vaccines. This method may serve as a comparative method with regard to standard determinations of immunogenic activity by WHO or OIE recommended techniques.

b) For the purpose of comparing the immunogenic activity and antigenic activity of vaccines an improved method, FAVN, was introduced for evaluation of the specific antibody response. An optimised evaluation of the ELISA test intended for quantification of both rabies antibodies and rabies antigen was developed and introduced.

Observations were carried out to determine the influence of haemolysis of blood samples on the results of quantitative determination of rabies antibodies by the ELISA test.

We evaluated the potential immunosuppressive influence on the immune antirabies response in laboratory animals. The effect of *Encephalitozoon cuniculi* was chosen as a model example of immunosuppressive influence on the level of protection induced by rabies vaccination.

Orientation of research in the near future:

– Improvement of the method for the evaluation of immunogenic activity of injection rabies vaccines by making it more objective through the following steps:

a) Using autochtonous street strains of the rabies virus as challenge strain in the process of evaluation of the effectiveness of rabies vaccines (genotypes of these strains may differ from those of the vaccination strain).

b) Using identical strains of rabies virus in a test in which both the vaccination strain of the tested vaccine and the challenge strain are of the same genotype (homologous test system).

c) Using rabies virus strains of different origin but of the same genotype (heterologous test system).

Development of an innovatory, improved, complex areactogenic adjuvant intended for the potential effectiveness of veterinary (and also rabies) vaccines. Validation of its safety and effectiveness.

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FOLIA VETERINARIA, 48, 1, Supplementum: S51—S52, 2004

HORMONAL DISBALANCES AND ENVIROMENTAL FACTORS RELATED TO ALLERGIC DISEASES IN DOGS — RISK ARISING FROM SYMPTOMLESS CARRIERS OF FUNGAL DISEASES AMONG COMPANION ANIMALS AND THE POTENTIAL CONSEQUENCES FOR THE HUMAN POPULATION

Projects VEGA SR, No. 1/7028/20 and No. 1/9012/02): Duration of the projects: 1st. – from: 01/2000 to 12/2002; 2nd – from 01/2002 to 12/2004, principal investigator: Prof. M. Kozák, DVM, PhD, I Internal Clinic of UVM Košice

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Investigations of hormonal disbalances and environmental factors related to dogs' allergic diseases conducted within the work on the project proved that environmental factors affecting population of dogs in the Košice region caused problems in these animals that were similar to those observed in humans (1, 2, 3). Allergen diagnostic methods proved that 81 % of the examined atopic dogs suffered from allergic diseases. The study of endocrinopathies showed an age-related dynamics of serum T3 and T4 in dogs in dependence on seasons. Homoeostatic observations showed a statistically significant decrease in creatinine concentration in dogs with low level of thyroid hormones and a decreased level of leukocytes in these dogs (4, 5, 8). Concentrations of triglycerides, cholesterol and total lipids were significantly increased in dogs with decreased levels of T3 and T4 although they remained in the reference range (4, 8). The subsequent stages of our study focused on interactive relationships between the function of the thyroid gland, adrenals and homeostasis, skin status, and the immune system of dogs. The knowledge obtained is usable in clinical practice in the field of diagnosis and therapy concerning the treatment of endocrinopathies and allergies in dogs.

The basic aim of studies related to the risk arising from occurrence of symptomless carriers of fungal diseases among companion animals and its potential consequences to human population originated from a real situation and the needs of veterinary small animal practice, particularly the clinical practice. We focused on the group of dermatophytic and micromycetic agents and their participation in skin diseases in dogs the incidence of which is still very high. We also pointed a study of the dermatophytes in dogs and the risk of human infection, to the specificities of their occurrence, course of diseases and diagnostics (6, 7, 9).

Orientation of research in the near future: Protection of human health through healthy animals is the basic idea of future research activities. We plan to monitor systematically the infestation, factors, and circumstances that are vital to the most frequently occurring target zoonoses, *salmonellosis, chlamydiosis, leptospirosis* and *toxoplasmosis,* in dogs and cats. Systematic screening will enable us to investigate their role in circulation and determine the most suitable ways for eradication, prevention and prophylaxis of these zoonoses under our conditions. After reconsidering the epidemiological seriousness and level of spreading in populations of dogs and cats a system of permanent control and elimination of *leptospirosis, salmonellosis, toxoplasmosis* and *chlamydiosis* may be introduced to keeping dogs and cats. A permanent *"Programme of veterinary-health care about dogs and cats"* will also be defined as one of vital contributions of the project.

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THE STUDY OF HEPATOPATHIES IN COMPANION ANIMALS

Project VEGA No. 1/804/0: Duration of the project: from 01/2001 to 12/2003,
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The first stage of the project investigated the potential use of a biochemical profile in the diagnosis of hepatopathies, particularly with regard to updating the values of biochemical parameters of the hepatic profile in healthy cats. Age dependence of the activity of enzymes (AST, ALT, ALP) in the serum of healthy young (6, 8, 12-month old) and adult cats was evaluated.

The age dependence of the activity of ALP was confirmed even in 12-month old animals. The dispersal of physiological values was determined also for other usable parameters (GMT, total bilirubin, urea, glucose, total lipids, cholesterol, triglycerides) including a parameter in the blood serum of cats (non-esterified fatty acids – NEFA), the reference values of which have not yet been determined by our laboratories. A method for the functional testing of liver based on determination of bile acids in the blood serum (after fasting – FSBA and after provoked feeding-postprandial – PPSBA) was developed and introduced into practice (4).

In the second stage of the project cats were observed for changes in haematological, biochemical and acid-base parameters induced in the short-term (12 hours) and in long-term (28 days) negative energy balance (1) and its influence on clinical symptoms and development of idiopathic feline hepatal lipidosis (2). The possibility of affecting these changes by administering a therapeutic dose of hepatoprotective substance has also been investigated.

Observation of the acid-base balance showed a statistically significant ($P<0.01$) shift in blood pH towards acidosis after 12 hours and significant change in HCO_3 and BE ($P<0.001$). These values continued to decline toward acidosis up to day 14 of the experiment when they became stabilised. A marked positive correlation was observed between the values of pH, HCO_3 and – BE ($r=0.9546$ and 0.9949, resp.) (5).

Orientation of research in the near future: The future research will focus on observation of correlation between the determination of the intensity of lipidosis based on histological bioptate and quantitative determination of fat in samples taken at laparotomy. Our modification of the method of evaluation of lipidosis findings will be compared with other diagnostic methods, using particularly non-invasive diagnostic techniques (3). In this respect we will observe the suitability of ultrasonography which allows us to characterise in a non-invasive way the diffusion changes in the liver parenchyma and to check the suitability of USG examination in correlation with histological changes in the liver. Our observations will be oriented on etiopathogenesis, clinical, biochemical, haematological, pathological-morphological and special diagnosis, therapy and prevention of various hepatopathies in various breeds of dogs of different age, sex, and use, and in cats and exotic birds.

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FOLIA VETERINARIA, 48, 1, Supplementum: S55, 2004

THE OCCURRENCE, SPREAD AND SELECTED ASPECTS OF THE ECOLOGY OF NEW, INVASIVE SPECIES OF FISH IN THE BODROG CATCHMENT BASIN IN THE EAST SLOVAKIAN TERRITORY

Project VEGA SR, No. 1/9209/02: Duration of the project: from 01/2000 to 12/2002, principal investigator: P. Košuth, DVM, PhD, Department of Infectious Diseases and Parasitology of UVM Košice

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The research is oriented on new, invasive species of fish that invade the eastern Slovakia *via* the river Tisa. The several species that have invaded our territory are species that could in several years reduce our original ichthyofauna (2, 3). If these new species, particularly the Amur (Chinese) slipper (*Percottus glenii*) were able to penetrate our carp farms they would cause an immense damage, much higher than that caused by the stone moroko (*Pseudorasbora parva*) that entered our rivers in the eighties of the past century (1, 4).

Orientation of research in the near future: Observation of movement of new, invasive species of fish into the rivers of the eastern Slovakia region, particularly those of the East Slovakian Lowlands. We refer particularly to the very dangerous species *Percottus glenii* and *Ictalurus melas.* Only additional field surveys will show whether additional species may be involved that try to move *via* the rivers Tisa, Bodrog and Latorica iconstantly into Slovak territory.

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FOLIA VETERINARIA, 48, 1, Supplementum: S56—S57, 2004

THE USE OF A GENE POOL OF AUTOCHTONOUS BREEDS FOR OBTAINING MORE RESISTANT ANIMALS INTENDED FOR ECOLOGICAL FARMING AND THE PRODUCTION OF BIOFOOD

Project VEGA SR, No. 1/8038/01: Duration of the project: from 01/2001 to 12/2003, principal investigator:
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The project investigates the potential use of the immunogenic properties of autochthonous breeds for obtaining productive animals with increased performance and resistance levels, suitable also for the harder conditions of risky areas. The possibility of the application of ecological breeding in some ruminants and poultry breeds was judged as well. Comparisons of examinated and actualized parameters of reference scale of physiological values of metabolic and immunological profile in selected hybrid combinations, small size populations of animals respectively, were performed.

The contribution is an attempt to regenerate the autochthonous brachycerous cattle – Carpathian red breed. Those are bred sporadically in the north-east part of the Slovak Republic around the Dunajec river, close to the Polish borders on the both sides of the Tatra mountains. Their registered breed is in Poland. In partial works some productive parameters in red cattle and their crossbreeds with brachycerous type – Angler breed (25 and 37.5 % blood ratio) were compared. In crossbreeds the percentual lipid content increased (4.12–4.28–4.30 %). Different values in percentual content of milk proteins were found (3.40–3.35–3.43 %). Variability of milk protein – kappa-casein indicate positive relationship to milk protein content (1, 2, 3). Association of kappa-casein gene pools to technological properties of milk are also known.

In the identification of the properties and signs of autochthonous poultry breed – Oravka breed (Or) besides the typing of productive parameters fractions of serum proteins and lipoproteins were analysed as well as an analysis of parameters in small size new gene pools of poultry – Araucana breed (Ar). The examinations were supplemented with the analysis of their synthetic lines (Or x Ar). In the comparison of hybrid combination mentioned with a commercial broiler hybrid ISA 220 the differences in carcass cutout value were detected (64.99 and 66.64 % in benefit to ISA 220). Besides this, in a comparison of the biochemical parameters of eggs of Araucana breed and layer hybrid breed (Shaver Starcross) it was determined that in Araucana breed during the weeks of laying period the weight of yolk as well as percentual ratio of yolk to eggs weight increases together with an increase of total egg weight (30.80–24.69 % and 89.28–96.24 %) (5). In the chicks of combined type good hatchability and resistance to harder breeding conditions, namely in areas of environmental risk are appreciated (4).

Orientation of research in the near future: Extend the existing results to foreign cooperation in monitoring the breed structure of autochthonous breeds in neighbouring countries and compilation of material for the database of protected breeds:

– to make the data of gene reserves sources available in online databases for breeders and breeders organisations,

– completion of the analyses of productive parameters widened to utilisation in areas of environmental risk and ecological breeding systems,

– in the synthetic lines the creation of bioproducts production targeting the selection of gene pools with lower levels of cholesterol and lipoprotein fractions, namely low density lipoproteins (LDL),

– broadening of knowledge about the non-specific immunocompetence of the autochthonous and small sized gene pools with the aim of their utilisation in more resistent and productively more efficient utility animal type creation.

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FOLIA VETERINARIA, 48, 1, Supplementum: S58, 2004

THE USE OF THE ATP METHOD IN FOOD HYGIENE INSPECTION

Project VEGA SR, No. 1/9011/02: Duration of the project: from 01/2002 to 12/2004,
principal investigator: J. Kottferová, DVM, PhD, Department of the Environment of UVM Košice

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At the present, the risk associated with the potential danger arising from contamination of food with harmful micro-organisms raises increased concern of the public. Producers have to pay increased attention to the biological cleanliness of production facilities and technological equipment. These activities are related also to the optimisation of sanitation programmes and procedures. A decisive factor in this process is the rapid obtaining of results which enables effective corrective measures to be taken before the operation even starts (3). The sensitivity of the methods used is also important. The most suitable appears to be the method based on detection of residues of adenosine triphosphate (ATP), the substance that occurs in all plant and animal cells. Contrary to conventional microbiological methods which confirm only the presence of bacteria and other micro-organisms and provide results some tens of hours later, the ATP method allows one to detect the presence of residual biological material within one minute. With regard to these facts the method of detection of ATP appears to be the only method of inspection of sanitation effectiveness that can comply fully with requirements of the HACCP (Hazard Analysis and Critical Control Point) concept (2).

The first stage of the project focused on the experimental validation of the ATP method and testing of its accuracy and sensitivity in comparison with other conventional microbiological methods under laboratory conditions (1). The results show that the method of determination of ATP is suitable for rapid verification of the effectiveness of sanitation in practice and is comparable with conventional microbiological method of determination of total viable count (TVC). The correlation of results ATP and TVC showed a significant number of occasions where there may be no bacterial contamination, but the presence of soiling is a threat to hygiene standards. Now, in the food industry, it is possible to test surfaces immediately after cleaning and to find out within minutes if the cleaning was succesful (3).

Orientation of research in the near future: The next stage of the project will involve introduction and validation of this method with regard to its capability in distinguishing individual types of ATP (total, free, somatic, and microbial ATP). Its suitability for monitoring of the level of hygiene and sanitation in selected milk and meat processing plants will also be investigated.

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FOLIA VETERINARIA, 48, 1, Supplementum: S59, 2004

RESIDUES OF ANTICOCCIDIAL DRUGS IN FOOD AND RAW MATERIALS OF ANIMAL ORIGIN

Project VEGA SR, No. 1/7027/20: Duration of the project: from 01/2000 to 12/2002, principal investigator: Prof. R. Cabadaj, DVM, PhD, Department of Food Hygiene and Technology of UVM Košice

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The aim of the scientific project was to observe the sensitivity of individual test microorganisms *Bacillus stearothermophilus* var. *calidolactis* C 953, *Bacillus subtilis* BGA, *Micrococcus luteus* ATCC 9341 and *Bacillus cereus* var. *mycoides* ATCC 11778, used in the screening of drug residues by microbiological methods, to the residual concentrations of amprolium, nicarbazin, furazolidone, monensin, lasalocid, sulphadimidine and sulphaquinoxaline, and to determine the minimum inhibiting concentrations (MICs) and the most sensitive test organisms for the anticoccidials tested.

According to our results (1) and in comparison with the MRLs established for these drugs, the microbiological methods can be used to obtain primary confirmation of the presence of monensin (MIC 0.001 mg.ml^{-1}, *Bacillus stearothermophilus* var. *calidolactis* C 953), *lasalocid* (MIC 0.001 mg.ml^{-1}, *Bacillus stearothermophilus* var. *calidolactis* C 953), *sulphadimidine* (MIC 0.01 mg.ml^{-1}, *Bacillus subtilis* BGA, pH 7.2), *sulphaquinoxaline* (MIC 0.001 mg.ml^{-1}, *Bacillus subtilis* BGA, pH 7.2), and *furazolidone* (MIC 0.001 mg.kg^{-1}, *Bacillus subtilis* BGA, pH 6.0) only. These drugs indicate the antibacterial activity and sensitivity of the test organisms. Amprolium and nicarbazin are chemical compounds and, therefore, more specific methods must be applied to detect their presence.

To detect the residues of both monensin and salinomycin in the tissues of broiler chickens after their experimental administration, a microbiological method with *Bacillus stearothermophilus* var. *calidolactis* C 953 and the four-plate method (Bo- gaerts and Wolf, 1980) were used. The sensitivity of the four-plate method to salinomycin residues was greater so they were detectable in the target tissues for four to six days depending on the dosage received (2). In the case of the screening of monensin residues in the tissues and blood sera of broiler chickens, more positive results were observed with the four-plate method. However, in comparison with the screening of test concentrations of monensin standard solutions, greater sensitivity with regard to detection limit of monensin was achieved using the microbiological method with *Bacillus stearothermophilus* var. *calidolactis* C 953. The residues were detected up to the 3rd day of the withdrawal period (3).

The monensin residues in the tissues and blood sera of broiler chickens up to day 3 of the withdrawal period were detected by high-performance liquid chromatography (HPLC) using pre-column derivatization with 2.4-dinitrophenylhydrazine at 300/390 nm simultaneously. Owing to a worse signal-to-noise ratio at 390 nm, UV absorption at 300 nm was used for quantification. The detection limit for monensin was 0.01 mg.kg^{-1}, and the recovery ranged from 70 to 98 %. The residues were detected up to the 2nd day of the withdrawal period (4).

The sulphadimidine residues in the whole eggs of laying hens throughout the six days of its oral administration and the fifteen days of the withdrawal period were detected by the HPLC method with DAD detection at 275 nm. The detection limit for sulphadimidine was 0.09 mg.kg^{-1} and the recovery range from 91 to 98 %. The residues were detected up to the 10th day of the withdrawal period.

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FOLIA VETERINARIA, 48, 1, Supplementum: S60, 2004

THE RESIDUES OF SULPHONAMIDES IN ANIMAL PRODUCTS DERIVED FROM POULTRY

Project VEGA SR, No. 1/0617/03: Duration of the project: from 01/2003 to 12/2005, principal investigator: Assoc. Prof. D. Máté, DVM, PhD, Department of Food Hygiene and Technology of UVM Košice

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Sulphonamide residues present a potential risk to human health. In order to protect the health of consumers, it is necessary to establish effective monitoring and testing programmes for the control of sulphonamide residues in foods of animal origin. The project is focused on the development of methods for the detection of sulphadimidine residues in animal products derived from poultry using the following:

1. microbiological agar diffusion methods for the detection and presumptive identification of sulphadimidine residues in animal products derived from poultry with the experimental design of the Four Plate Test (FPT), and utilisation of the inhibitory effect of para-aminobenzoic acid (PABA) as a confirmatory solution;
2. high-performance liquid chromatography (HPLC) and alternative methods of extraction with UV detection for the quantitative determination of sulphadimidine residues in animal products derived from poultry.

The *Codex Alimentarius* of the SR establishes the maximum residue limit (MRL) of 0.1 $mg.kg^{-1}$ for sulphonamides in foods of animal origin. The aim of the project is to develop the methods methodologically suitable for detecting sulphadimidine residues in animal products derived from poultry at or below the level of the MRL.

The objective of the first stage of the project is to evaluate the reference Four Plate Test (FPT) using a test micro-organism *Bacillus subtilis* BGA (Test agar pH 7.2; trimethoprim at a concentration of 0.05 $\mu g.ml^{-1}$) for the detection of sulphadimidine residues to determine the detection sensitivity of the method with regard to residual concentrations of the sulphadimidine standard. After determining the minimum inhibiting concentration (MIC) for sulphadimidine, the effects and interaction of three selected parameters (the pH value of agar medium, trimethoprim concentration, and the addition of dextrose to the agar medium) on increasing the detection sensitivity of the test micro-organism *Bacillus subtilis* BGA and on lowering the MIC for sulphadimidine will be studied. To identify the sulphadimidine residues, the method will be amended for one more plate with the addition of PABA at the concentration most suitable for identification of sulphadimidine residues at the levels of concern. Our results indicate (1) that the most suitable concentration of PABA for the identification of sulphadimidine residues was 20 $\mu g.ml^{-1}$. To improve the detection level for sulphadimidine, we raised the trimethoprim concentration to 0.15 $\mu g.ml^{-1}$ of agar.

The part of the first stage of the project is to develop the HPLC method for the quantitative determination of sulphadimidine residues by using two alternative methods of extraction (solid-phase extraction – SPE and matrix solid-phase dispersion – MSPD) with UV detection. Our investigation will focus on the development of simple, rapid, and reliable methods using the procedure mentioned for the treatment, purification, and extraction of samples and ensuring the maximum recovery and the lowest possible detection limit.

Orientation of the research in the near future: The aim of the second stage investigations (2004—2005) is to apply the analytical methods developed in the first stage of the project (2003—2004) in order to detect, identify, and determine quantitatively sulphadimidine residues in animal products derived from poultry.

The analytical methods will be examined on the layers with the recommended therapeutic doses of the preparation described above. During the fifteen day withdrawal period we will determine the residual concentrations of sulphadimidine in the muscle, edible internal organs (heart, gizzard, and liver), skin, fat, and eggs of the layers. Part of the experiment will be devoted on evaluation of the potential effects of biological matrices on the detection limits of the methods developed.

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FOLIA VETERINARIA, 48, 1, Supplementum: S61, 2004

THE INFLUENCE OF ANTIOXIDANTS APPLIED *in vivo* AND *in vitro* ON THE QUALITY OF MEAT SLAUGHTER ANIMALS AND MEAT PRODUCTS

Project VEGA SR, No. 1/8237/01: Duration of the project: from 01/2001 to 12/2003, principal investigator: Assoc. Prof. P. Turek, DVM, PhD, Department of Food Hygiene and Technology of UVM Košice

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In the framework of the solution of our project, the method of detection the malondialdehyde as a main product of polyunsaturated fatty acids oxidation by high performance liquid chromatography was developed (1, 4). The use of this method for detecting oxidative products in meat and meat products is advantageous because of specificity, speed, simplicity and cost effectiveness.

Oxidative degradation products were estimated in raw material that was used for meat products manufacturing and also had influence on the oxidative degradation of fats in ready-made meat products dependent on manufacturing technology, methods of storage and packaging (2). In the experiments performed, the antioxidative effect of rosemary powder FlavorGuard P (Christian Hansen, Denmark) on the stability of fats in meat and meat products was evaluated (3). This rosemary powder was added to the feeding formulas used in broilers and as an additive directly into the meat products. The antioxidative effect of rosemary powder was increased in combination with ascorbic acid and vitamin E. Positive effect was approved also by sensory analyses of the meat products.

Orientation of the research in the near future: Many herbs and spices are known as antioxidants, which have been intensively investigated in recent years. The future prospects in this field will be preparation of extracts of herbs as a potential source of natural antioxidants. Extracts will be used for the suppression of oxidative processes in meat and meat products during different conditions of thermal treatment and storage.

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FOLIA VETERINARIA, 48, 1, Supplementum: S62, 2004

THE RESIDUES OF ANTIMICROBIALS IN FOOD AND RAW MATERIALS OF ANIMAL ORIGIN

Project VEGA SR, No. 1/7021/20: Duration of the project: from 01/2000 to 12/2002, principal investigator: Assoc. Prof. J. Nagy, DVM, PhD, Department of Food Hygiene and Technology of UVM Košice

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The presence of veterinary drug residues in foods of animal origin intended for human consumption has become a subject of continuous concern on the part of health and veterinary authorities, food hygiene, research programmes and the public in general.

High performance liquid chromatography (HPLC) methods for the detection of tylosin and tetracycline residues in food of animal origin (meat, internal organs, eggs, milk, and honey) were developed (1, 2). The sensitivity of HPLC, microbiological methods and commercial screening tests routinely used in the practice were compared by means *in vitro* and *in vivo* experiments (3, 4).

Orientation of the research in the near future: The work on the project (Project VEGA SR, No. 1/0618/03) will include the isolation of micro-organisms from the carcasses of slaughter animals that were administered various drugs before slaughter or from foods of animal origin. The resistance to antibiotics will be compared among the strains isolated from slaughtered animals treated with different drugs, as well as from the control group (animals without any treatment). The influence of technological procedures used in the production of food on the sensitivity of target micro-organisms to antibiotics will also be studied.

We will look for correlations between the bioavailability of drugs and residue levels depending on the respective drug, the methods of administration, the drug dose, age of animals, and the liquid used for its administration. In this study, the target micro-organisms (the most frequent causative agents of food-borne illness or the most dangerous bacteria), such as *Salmonella* sp., *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes* will be tested for their sensitivity to various medicines.

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FOLIA VETERINARIA, 48, 1, Supplementum: S63, 2004

THE DECREASE IN HEALTH AND TECHNOLOGICAL RISK TO MILK ENSURING THE HEALTH SAFETY OF MILK FOR HUMAN CONSUMPTION AND OF MILK PRODUCTS

Project VEGA SR, No. 1/9018/02): Duration of the project: from 01/2002 to 12/2004, principal investigator: Prof. O. Burdová, DVM, PhD, Department of Hygiene and Technology of Food of UVM Košice

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Dairy cow mastitis increases PSB, free fatty acids and the activity of lipase which becomes more intensive in milk with PSB exceeding 400,000 per millilitre and with the storage of raw milk longer than twenty-four hours at temperatures exceeding 5 °C.

The total content of proteins does not necessarily change but a decrease in the synthesis of casein (changes in casein fractions, decrease in as and β-casein and increase in γ-casein) and serum proteins, namely β-lactoglobulin and α-lactoalbumin and an increase in the content of immunoglobulins is observed. Both the increase in pH and decrease in Ca (by 36 %) and Mg (by 20 %) decrease the time of thermal denaturation by as much as 10 % which decreases the thermostability of proteins.

The lactose content declines while the content of chloride ions and total minerals increases but the level of Ca, K, Mg, P and citrates decreases. The increased number of somatic cells is accompanied by an increase in the activity of enzymes (protease, lipase) which results in hydrolysis of milk fat, an increase in free fatty acids and a decrease in the storage life of the products.

The fat content is not necessarily decreased but there are changes in the size of fat globules, the proportion of short-chain fatty acids increases as does the amount of unsaturated fatty acids (1).

Orientation of research in the near future:

– To validate the possibilities of suppression of *Staphylococcus aureus* in some milk products during their processing by means of a bactericin substance produced by *Enterococcus faecium* CCM 4231 which is a small, thermostable hydrophobic substance with a wide antimicrobial action. Its inhibitory effects against *Listeria* spp. and staphylococci have been confirmed in some milk products but also in fermented salami (2).

– To study the effect of bactericins and potential use of their antimicrobial activity in the process of production and ripening of some milk products (fermented milk products, cheeses, and similar), particularly of class II bactericins produced by a wide group of G^+ bacteria focusing on the anti-staphylococcal activity of the enterocin produced by the strain *Enterococcus faecium* CCM 4231, with the possibility of their experimental application in decreasing the biological risk resulting from milk and milk products (3). The experimental part will be directed towards the verification of the potential application of purified bactericin preparations in increasing the quality, shelf life and microbial safety of milk products.

– To define optimum conditions and methods for the determination of proteolytic and lipolytic activity of psychrotrophic microflora during the storage of both raw milk and final products.

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FOLIA VETERINARIA, 48, 1, Supplementum: S64—S65, 2004

THE OCCURRENCE AND SURVIVAL OF ENTEROPATHOGENIC *Escherichia coli* O157:H7 IN MILK AND MILK PRODUCTS

Bilateral scientific project between the Slovak Republic and Greece Gr/Sl/01-3: Duration of the project: from 05/2001 to 05/2004, principal investigator: Prof. R. Cabadaj, DVM, PhD, Department of Food Hygiene and Technology of UVM Košice

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The aim of the project is to monitor the occurrence of enterohaemorrhagic *E. coli* O157:H7 in raw milk and selected milk products. As this pathogen belongs to the family Enterobacteriaceae, it can be easily devitalised by any type of heat processing including pasteurisation. Therefore, its presence can be expected mostly in foods which have not been exposed to thermal treatment (1).

In the first phase of this project, samples of raw cow, sheep, and goat milk, raw sheep cottage cheese, as well as bryndza-cheese were taken from 32 dairy farms in the region of East Slovakia and examined microbiologically. Sampling, transportation, and preparation of samples for microbiological examination, as well as the examination itself complied with the government regulations and standards for microbiological examination of food, milk and milk products. Identical procedures apply in both partner countries (The Slovak Republic and Greece).

Sixty *E. coli* isolates were subjected to biochemical confirmation tests with the help of the ENTERO 16 and ENTERO 24 kits (2). Furthermore, the ability and type of haemolysis, resistance to selected drugs as well as sensitivity to two sanitising agents commonly used in the milk industry were determined for all *E. coli* isolates. According to the results, high counts of α-haemolytic *E. coli* strains in bryndza-cheese make this product open to suspicion of containing verotoxinogenic *E. coli* strains (e. g. serotype O157:H7). These can cause serious infections manifested by haemorrhagic colitis or even by the so-called haemolytic-uraemic syndrome.

The results of microbiological examination aimed at the detection of the family Enterobacteriaceae in the samples of cottage cheese and bryndza-cheese showed the presence of a wide range of enterobacteria in both cheeses (2). In samples of cottage cheese, *Citrobacter* sp. (33.5 %) and *E. coli* (30.2 %) were found in the highest proportions (4).

On the other hand, the percentages of *Enterobacter* sp. (2.8 %) and *Pantoea* sp. (1.6 %) were very low. With regard to bryndza-cheese, the counts of *Kluyvera* sp. (62.32 %) and *Enterobacter* sp. (17.14 %) were highest. The occurrence of *Pantoea* sp. (0.05 %), *Serratia* sp. (0.08 %) and *Citrobacter* sp. (0.31 %) was negligible (4). *Listeria monocytogenes* were found in 19 % of raw cow milk, 50 % of sheep milk and 10 % of sheep cottage cheese samples (3).

Currently, there is no government regulation for the isolation of *E. coli* O157:H7 serotype from food in The Slovak Republic. The State Veterinary Administration has adopted an official procedure that is used for this purpose in the European Union. This method is based on a selective enrichment of each sample in the broth with novobiocin followed by an immunomagnetic separation using anti-*E. coli* O157 dynabeads. Finally, the concentrated material is spread onto the surface of two selective solid media with the preference of fluorogenic or chromogenic ones. Owing to this project, this procedure has been introduced successfully into practice in our microbiological laboratory. Last spring our department started an intensive investigation of raw milk and milk products in order to map the epidemiological situation throughout The Slovak Republic.

Orientation of research in the near future: The viability and behaviour of individual *E. coli* O157:H7 strains under various conditions (temperature, pH, a_w) will be observed. These conditions will simulate the individual technological steps that are used during the processing and storage of milk and dairy products. The results obtained will serve as a basis for further investigation in the field of epidemiology (the comparison of isolates between both participants), health (the testing of strains for antibiotic-resistance and the production of verocytotoxin), and technology (the survival of *E. coli* O157:H7 isolates under various conditions which imitate the technologies used in milk processing and production of dairy products). They should contribute to the recovery of *E. coli* O157:H7 from food minimising in this way the possible health risk to the consumer.

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FOLIA VETERINARIA, 48, 1, Supplementum: S66—S67, 2004

THE POSSIBILITY OF THE RAPID DETECTION AND PREVENTION OF STAPHYLOCOCCI ENTEROTOXICOSES CAUSED BY MILK AND THE MILK PRODUCTS OF DIFFERENT ANIMAL SPECIES

Project VEGA SR, No. 1/7038/20: Duration of the project: from 01/2000 to 12/2002, principal investigator: Assoc. Prof. E. Dudriková, DVM, PhD, Department of Food Hygiene and Technology of UVM Košice

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Staphylococcus strains were isolated from samples of raw cow milk, sheep milk and milk products (pasteurised and long shelf-life milk, sweet and sour cream, butter, dried milk and sheep lump cheese).

A modified Ouchterlony's immunodiffusion precipitation method for detection of staphylococci enterotoxins in milk and milk products has been introduced into laboratory practice. Production of type A-D staphylococci enterotoxins by *S. aureus* strains isolated in our laboratory from cow milk and milk products was not confirmed. A method for isolation of DNA and PCR methods for detection of staphylococci enterotoxins produced by *S. aureus* strains isolated from milk and milk products were tested. The results obtained corresponded fully with the method of immunodiffusion precipitation.

Non-enterotoxigenic strains of *S. aureus* were isolated from cows with chronic inflammation of the mammary gland. They were present in milk samples even on day 9 following the application of tylosin (once a day for five days) (1).

The influence of the environment on the occurrence of individual representatives of the genus *Staphylococcus* in raw sheep milk was confirmed. The sources of contamination of raw cow and sheep milk and milk products with staphylococci were specified. Production of type A-D staphylococci enterotoxins by the staphylococci strains isolated from raw sheep milk (*S. aureus, S. capitis, S. auricularis, S. simulans, S. epidermidis, S. cohnii*) was not proved (2).

Changes in the development of staphylococci microflora (*S. aureus*) in lump sheep cheese made directly at the sheep farm from non-pasteurised sheep milk and stored at various temperatures were observed. An increase in the numbers of *S. aureus* bacteria was observed in cheese sample stored for three days at 21 °C and then at 14 °C during the first two day storage of cheese under the sheep farm conditions. No presence of staphylococci that produce type A-D staphylococci enterotoxins was observed in lump sheep cheese made from non-pasteurised sheep milk under sheep farm conditions (3).

It has been proved that noxious insects of the order DIPTERA, e.g. housefly (*Musca domestica*), mosquito (*Culex molestus*), cheese skipper (*Piophila casei*), Indian meal moth (*Plodia interpunctella*), German cockroach (*Blatella germanica*), and woodlouse (*Onyscus asellus*) can transmit *S. aureus* in production facilities producing milk for human consumption, dried milk, yoghurts, cream, butter and bryndza-cheese (4).

We prepared a proposal for primary milk production intended for practice consisting of a flowchart of production of raw cow milk within proper manufacturing practice or hazard analysis of risk and critical control points (HACCP) system with identification of critical control points that includes a description of microbiological and chemical risk, critical limits, monitoring, corrective measures and system verification (5).

Orientation of research in the near future: On the basis of defining the sources of contamination of raw materials and food of animal origin, plant-type food and vegetable additives, the future study will focus on the relationship between the origin, biotype, and potential enterotoxigenicity of strains isolated from production premises. The study will focus on bacteria indicating insufficient sanitation (*Staphylococcus aureus, Escherichia coli*), sporulating bacteria (*Clostridium* spp., *Bacillus* spp.), and micromycetes (*Geotrichum* spp., *Penicillium* spp., *Mucor* spp.). The output for practice will consist in introduction of an objective evaluation of microbiological and chemical quality of raw materials and food of animal origin from the point of view of hygiene and food safety. Preventive and corrective measures for decreasing the risk resulting in contamination of raw materials and food of animal origin will be suggested within the context of correct manufacturing practice and the system of hazard analysis of risk and critical control points (HACCP).

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FOLIA VETERINARIA, 48, 1, Supplementum: S68, 2004

SUSTAINING ANIMAL HEALTH AND FOOD SAFETY IN ORGANIC FARMING (SAFO)

FP5, Key Action 5.1.1, Project No. QLRT 2001-02541
Project co-ordinator: Mette Vaarst, Danish Institute for Agricultural Sciences, Denmark
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Duration of the project: from 02/ 2003 to 10/2004

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In Europe, organic livestock production has experienced rapid growth in the past decade. This development has not been without problems. Recommended practices in the European Organic Livestock Standards, such as closed herds and flocks and improved health security on farms, also include extensive production systems (e. g. free range production) that expose livestock to increased disease challenge. An EU-funded Concerted Action project, *"Network for Animal Health and Welfare in Organic Agriculture (NAHWOA)"* identified in 2001 that organic livestock production faces major challenges with regard to harmonisation and successful integration of organic animal husbandry into the whole organic production system.

Common EU standards for organic animal production were implemented only some eighteen months ago (EU Regulation 1804/1999). The implementation process will be an ongoing effort to harmonise the standards under widely differing circumstances and farming conditions in the member states. Significant diversity between farming systems, e.g. between the southern and northern European countries, should be taken into account in developing farming systems that all comply with the EU standards, but are in harmony with their geographic and cultural localities. Furthermore, the introduction of candidate member states from Central and Eastern Europe into the EU will increase the need to harmonise standards and make them practically applicable in these countries.

Organic farming in the Slovak Republic has to comply with the Act. No. 224/1998 of the Code about ecological agriculture and production of biofoods, referred to in the Act No. 415/2002 of the Code and the Decree No. 3259/1999-100 of the Ministry of Agriculture of SR. They specify the rights and obligations of physical and legal persons who produce bioproducts, collect herbs or their parts or process them and produce biofoods and put them on the market. They cover all aspects necessary to ensure high quality of bioproducts that reach the consumer. The Association of Friends in Organic Farming in SR updates regularly website information about organic farms and bioproducts. At the present, organic farms cover about 60,000 hectares of land (of this about 64.5 % are pastures, 35 % arable land, and the rest are orchards and vineyards).

The ultimate objective of the project: to improve food safety and animal health in organic livestock production systems in existing and candidate member countries of the European Union through exchange and active communication of research results and conclusions between researchers, policy makers, farmers and the wider stakeholder community, including consumers.

The main intermediate objectives are:

– to identify important food quality characteristics linked to organic products, and improve food quality, including food safety with regard to zoonoses, drug residues and the development of antimicrobial resistance in the food chain,

– to develop strategies for implementing and harmonising organic livestock production standards in existing and candidate member countries,

– to improve the interaction between researchers, farmers, certification bodies and policy makers in order to guarantee the development of organic livestock standards that are driven by inputs from all stakeholders in the EU.

Orientation of research of Partner 12:

– to investigate the problems related to environmental pollution with respect to disposal of wastes from organic farms in dependence on season, climate conditions and the size of herd,

– to obtain information about survival of selected groups of bacteria important from the point of view of protection of animals and the environment in relation to the method of housing,

– to identify potential sources of risk to animal health and safety of products and suggest measures for improvement.