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The 18th Conference about Laboratory Animals organized by the Czech Laboratory Animal Science Association (SVLZ) was held in Hnanice near Znojmo in the South Moravia, May 5 – 7, 2015 in hotels Happy Star and Vinice. A total 78 of scientists, experts of veterinary administration, animal science or lawyers participated in the meeting. Most papers were devoted to welfare and laboratory animal protection, other presentations were reported new experimental methods or data from experiments both in animals or new alternative models. The amended regulations and laws at the care of laboratory animals were presented, too.

EFFECT OF *SILYBUM MARIANUM* FRUIT EXTRACT ON THE FATTENING PERFORMANCE AND THE HEALTH STATUS OF BROILER RABBITS

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Milk thistle (*Silybum marianum*) seed and fruit extract contains silymarin (large number of flavolignans, including silybin, isosilybin, silydianin and silychristin) with hepatoprotective and canceroprotective properties, neurodegenerative and neurotoxic repressing functions. The aim of this study was to evaluate the influence of *Silybum marianum* fruit extract (Silyfeed[®]Basic supplement) on the growth of broiler rabbits and their health status. There were 150 HYL A broiler rabbits divided to three groups in the private farm, with some incidence of coccidiosis and other diseases. Animals were fed by a standard diet for rabbit fattening without any supplement (group I), and with the supplement of 0.2 % Silyfeed[®]Basic for group II and 1 % for group III. The experiment started at 42 days of rabbit age and finished by the achievement of 2600 g of live weight. There were not found any statistical significant differences between experimental and control group in both parameters – the growth performance and the carcass yield. The morbidity and the mortality were significantly lower in the group III (1 % of Silyfeed[®]Basic). The results show that the *Silybum marianum* fruit extract supplemented in the amount of 1 % could be a suitable substitution for chemical drugs commonly used in broiler rabbit fattening.

BENEFICIAL EFFECTS OF PROBIOTIC BACTERIA ON *TRICHINELLA SPIRALIS* INFECTION IN MICE

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A protective effect of probiotic and bacteriocin-producing bacterial strains on *Trichinella spiralis* infection, a parasitic zoonosis, was examined in murine model as a new therapeutic strategy using probiotics to control parasitic zoonosis. A link between administration of probiotic bacteria, immune effectors induction, and parasite elimination was studied. Gut microbiota represents a relevant factor that may strongly interfere with the pathophysiology of parasitic infections, determine the parasite survival and the outcome of parasitic infections. Therefore probiotics can play an important role in reducing the pathogenicity of many parasites. Probiotic strains with a health benefit (e.g. lactobacilli, bifidobacteria, enterococci) are able to inhibit, displace and compete with pathogens, and enhance mucosal barrier activity, although these abilities are strain-dependent (Sun *et al.* 2013). The most interesting thing is their property to regulate the polarization of naïve immune system by skewing it away from Th2 toward Th1 responses, and thus promoting cell mediated immunity that can be used in therapy of allergies (Shida *et al.* 2002); or they stimulate the development of Th2 type with increased antibody response and can be an appropriate approach for treating inflammatory gastrointestinal disorders (Shida *et al.* 2011, Zhao *et al.* 2013). Nematode *Trichinella spiralis* has been chosen as a model parasite to verify antiparasitic and immunomodulatory properties of probiotic and bacteriocin-producing bacterial strains. *T. spiralis* causes an intestinal and tissue disease – trichinellosis characterized by the enteritis (induced by adult worms) and the inflammation with degenerative changes in the skeletal muscles (induced by larvae). The pathology of trichinellosis is particularly a reaction to the initial inflammatory response during the intestinal phase and to the subsequent allergic and inflammatory responses during larval

migration and invasion of the host muscles (Aranzamendi *et al.* 2013). Pathogenicity of *T. spiralis* is higher than other intestinal parasites due to the high production of newborn larvae (Pozio *et al.* 1992) and a strong immune response of the host (Pozio *et al.* 1993, Bruschi *et al.* 1999, Morales *et al.* 2002). Immunomodulatory properties and antiparasitic effect of probiotic and bacteriocin-producing bacterial strains were studied in model animals – mice (inbred strain BALB/c) whose the gut and immune system are similar to human organism in parasite *T. spiralis* infection. The experimental protocol was approved by the Animal Care Committee of the Institute of Parasitology SAS and the State Veterinary and Food Administration of the Slovak Republic (No. Ro-4296/12-221d). Bacterial strains (Institute of Animal Physiology SAS, Košice) were administered to mice daily *per os* at a dose of 100 µl (10⁹ CFU/ml in Ringer's solution). Two groups of bacteria will be examined, bacteriocin-producing strains with probiotic properties (*E. faecium* EF55 – chicken isolate, producing an antimicrobial proteinaceous substance enterocin (Ent) 55; *E. faecium* AL41 – environmental isolate, producing a new enterocin type Ent M; *E. faecium* 2019-CCM7421 – rabbit isolate, producing Ent 2019) and probiotic strains (*L. fermentum* AD1-CCM7420 – canine isolate; *L. plantarum* 17L/1 – isolate from stored sheep cheese). The parasitic infection was done *per os* with *T. spiralis* larvae on the 7th day of treatment. The parasite, originally obtained from the Trichinella Reference Centre in Rome, is passaged in outbred ICR mice at the Institute of Parasitology SAS. The infective dose was 400 larvae *per mouse*. A protective effect against parasite worm burden in the intestine (reduction cca 40 %) was observed only in mice treated with enterococci (*E. faecium* EF55, *E. faecium* AL41, *E. faecium* 2019-CCM7421). Stimulation of the host immune response (proliferative activity of T cells, CD4 subpopulation, metabolic activity of macrophages) resulted in reduction of muscle larvae with the highest efficacy (reduction cca 70 %) in mice treated with *E. faecium* AL41, followed by *L. fermentum* AD1-CCM7421 and *L. plantarum* 17L/1 treatment. The results indicate that examined probiotic strains might provide a strain-specific protection against parasites not only in the gut, but also in host tissues. Immunological interactions between probiotic strains, parasite and host cells need to be investigated more in details. The obtained data contributed to a strain-specific characterization of immunogenic properties of tested bacterial strains as promising candidates for prophylactic or therapeutic immunomodulation of the host with trichinellosis.

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CHARACTERISTICS AND FREQUENCY OF DETECTION OF MNV VIRUS

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Murine norovirus is small non-enveloped RNA virus belong to the family Caliciviridae. This gastrointestinal virus is the single most widespread pathogen found in research mouse colonies worldwide. Murine norovirus was first described in 2003. A few serotypes MNV 1-4 are known today. Duration of infection and disease manifestation depending on the mouse strain. In immunocompetent strains, MNV-1 infection does not induce clinical signs and is associated with mild histopathological alteration. Infection can cause lethal disease in certain immunodeficient strain with defect in the innate immune system. According to literature, MNV positivity varies by tens of percent. Our data for the last 5 years show percent range of MNV positive animals from 15.57 % to 20.68 %. Since 2014, when MNV virus is a part of all serological profiles, the percentage of MNV positive samples increased to 21.77-23.27 %.

DIAGNOSTICS OF PNEUMOCYSTIS CARINII IN LABORATORY RATS

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Pneumocystis carinii is a major opportunistic pathogen which has been found in the lungs of a wide variety of mammals including laboratory rats. It is common causative agents of idiopathic lung lesions in immunocompetent rats and *Pneumocystis* pneumonia in immunocompromised hosts. According FELASA recommendation 2001 *P. carinii* was diagnosed on the bases of lung lesions found during the autopsy. In 2013, we examined twelve laboratory rats using IFA, PCR and histopathology. Nine samples of serum were found positive by IFA, nine samples of DNA isolated from lungs were found positive by PCR and two lung samples were found positive by histopathology. In total eleven rats were positive on *P. carinii*. According new FELASA recommendation from 2014, we put diagnostics of *P. carinii* on the Annual programme. From June 2014 to the end of December 2014, we examined fifty-five rats using IFA and PCR. Twenty-four of them were positive by IFA and twelve by PCR. In total twenty-eight rats were positive on *P. carinii*. Prevalence of *Pneumocystis carinii* is high in laboratory rats which could be a problem when immunocompromised animals are used.

ETHOLOGICAL STUDY OF ENRICHMENT IN MARMOSETS (CALLITHRICIDAE) IN ZOO HLUBOKÁ

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Environmental enrichment helps to improve mental and physical state of animals in captivity. It also supports their active behavior and tries to reduce stereotypical behavior to which these individuals kept in captivity have tended to. This paper was created in the ZOO of Hluboká nad Vltavou – Ohrada. There has been chosen four species of marmosets and tamarins research, the whole amount consists of 13 individuals. The first group was composed from a male and two females of Pygmy marmosets. The second one was made up of two females of Golden lion tamarin and the third one was consisted of the family of Cotton-top tamarins, composed from a male, two females and two young ones animals (a male and a female). The last observed species was Emperor tamarin which has the zoo in numbers of a male, a female, and two young ones to who haven't been distinguished the sex yet. The main aim of the Thesis was to summarize the monkey's behavior during the period of enrichment and without it. Furthermore, the goal was to find out whether the given enrichment changed the behavior of individuals. These two components were given to monkeys e.g. a carved out melon and a bamboo ladder. We are speaking about the

enrichment based on consuming food. There was find out that after the statistic processing of the results, all observed animals were influenced and enriched by the given "toys".

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BACHELOR GROUPS OF PRIMATES (SUPERFAMILIA *CERCOPITHECOIDEA*) IN CAPTIVITY

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This paper deals with questions of primate breeding focused on the Cercopithecoidea superfamily in human care, especially solving the problem of breeding more males of the same species in a breeding station. Following the observation, there are proposed certain solutions and evaluated their positives and negatives. The observation proceeded among the macaque (*Macaca silenus*) group in the zoological and botanical garden in Pilsen, where the contraception was used, and in the centre for animal rehabilitation and education for chacma baboons in South Africa, where the males were separated by mechanical barrier. The next theoretical, unobserved, possibility is the euthanasia of unnecessary animals. The monitoring proved that the contraception method has not already been examined by sufficient number of tests. Although the male aggressiveness against each other and against females was reduced but there was a wrongful conception of one female. After the implant removal the male has not come back to the ability of bearing descendants. Compared to the contraception method, the mechanical barrier method prevented males from the fights, injuries and wrongful conception and did not anyhow limit the males in their reproductive future. Indeed it is quite demanding of space and realization. Euthanasia as a possible solution with excess of males is considered in some species by appropriate authorities, however due to the fact that it is irreversible and radical solution; its use should always be considered and cannot be recommended in general.

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A COMPLETE HEALTH MONITORING OF LABORATORY ANIMALS – ADVANTAGES AND DISADVANTAGES

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The recommendations for the health monitoring (HM) of laboratory animals have been created from the 50th years of last century. The first recommendation for breeding/experimental facilities in Europe was published by FELASA (Federation of European Laboratory Animals Associations) in the year 1991 and later 2001, 2014. The recommendations are focused mainly to pathogenic viruses, bacteria, parasites with higher incidences in European facilities. This fact is for example evident from positive results registered in our laboratory from all examined mice sera in the year 2010, 2012 and 2014. A detection of antibodies against *Murine norovirus* was in space 18-20 %, *Mouse hepatitis virus* 2.39-3.91 %, *Mouse parvovirus 1+2* in space 0.72-5.25 %. The findings of positivity at rats: *Kilham virus* 2.48-9.57 %; *Sialodacryoadenitis virus* 1.45-10.08 %, *Pneumonia virus of mice* 0.28-9.6 %, *H1 (Toolan) virus* 1.12-6.28 %, *Rat minute virus* 1.12-3.8 %, *Mycoplasma pulmonis* 1.16-5.24 %. Other tested viruses at mice and rats did not exceed 1 % of positivity. An importance of bacteriological findings at HM is emphasized with regard to different used methods and a recent requirements at using of transgenic animals and microbiome study. Complete HM should be focused in places where is a high probability of bacteria occurrence and all findings should be presented: all pathogens, opportunistic and potential pathogens and usual microflora. The presence of usual microflora mainly from gut can inform about bacterial disharmony. The complete HM has a few advantages by comparison with examination of collected samples only: evaluation of animal clinical status, appreciations of organs *in situ*, immediate bacterial cultivation and detection of parasites, identification of pathological lesions with additional sampling including histology, complete report for every individual animal. The disadvantages of complete HM are usually presented: euthanasia of animal, transport of animal, higher expenses.

IN VIVO TESTING COMBINATION OF DRUGS WITH THERAPEUTIC EFFECT AGAINST *MYCOBACTERIUM TUBERCULOSIS*

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Laboratory mice were infected with *Mycobacterium tuberculosis* intranasally (*i.n.*). This administration allows safe application of infectious organisms at accurately defined quantity of microorganism cells. Infectious process induced by such way, simulate natural transmission by aerogenic route. Therapeutical substances selected for the testing (isoniazid, rifampin and pyrazinamide) were administered orally by gavage or by drinking bottle from 1 to 3 months. All conditions were performed according to the law No. 246/1992 Sb. and related regulations. Manifestation of proceeded infection was observed during the experiment. Tissue samples, spleen and lungs, for determination of CFU (colony forming units) were taken after euthanasia.

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A BRIEF HISTORY OF A GNOTOBIOTIC PIG MODEL IN IMMUNOLOGICAL RESEARCH

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Gnotobiotic animals with their simple and defined microbiota are suitable models to study host × microbiota interactions. The pig is due its epitheliochorial type of placentation and anatomical and physiological similarities to the human frequent model in biomedical research. The pioneer attempts to obtain and rear germ-free piglets were done in the USA and later in the former Czechoslovakia at the

beginning of the second half of the last century. Methods of obtaining germ-free piglets were developed and improved from semi-sterile approaches to aseptic surgery techniques. Different materials and isolators as stainless steel, transparent plastic and fiberglass were tested and introduced. Renaissance of interest in gnotobiotic animal models has been dated since the beginning of this century mainly due to new methods of study of microbiota – e.g. PCR, high-throughput RNA analysis and next generation sequencing that again attracted attention to study of microbiota composition and importance. The gnotobiotic pig has been highly required animal model of many international collaborations with more than sixty years of its tradition.

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CEREBROSPINAL FLUID COLLECTION TECHNIQUE IN LABORATORY ANIMALS

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The aim of the lecture was to present a simple technique for efficient collection of cerebrospinal fluid (CRF) from cisterna magna of adult rats, guinea pigs and rabbits. The successful CRF collections are necessary for assessing CNS penetration and distribution of new chemical entities; it is utilized as a biological medium in human medicine. Concerning the veterinary practice, the CRF collections are also used for diagnosing neurological diseases caused by both infection agents and brain damage. Six Wistar male rats and six male guinea pigs weighing 300-400 g were anesthetized by ketamine (50 mg/kg) and xylazine (10 mg/kg) intraperitoneally. Likewise, six male rabbits weighing 3-4 kg were anesthetized by ketamine (40 mg/kg) and xylazine (4 mg/kg) in the same manner. Regarding the necessity of repeated CSF collections from surviving animals, we used the following method: Immediate commencement of anesthetization, the fur was clipped over a portion of the caudal head overlying the cisterna magna of the animals. Thereafter, the animal was held manually so that the occipital bone was almost horizontal to the table and the rest of the body was lying at about 90 degree angle to the head. A depressible surface with the appearance of a rhomboid area between occipital protuberance and the spine of the atlas becomes palpable. In this way, the position for the collection site was identified. After disinfection of collection site, the 27 or 23 needle was inserted into the soft rhomboid surface on the scalp. As the needle passed through dura mater into cisterna magna, a decrease in resistance was perceptible, which confirmed the presence of the needle in the cisterna magna. Then by a gentle aspiration, the non-contaminated CSF was drawn into a 1 ml syringe. In case of the terminal CSF collections, a small incision was made in the skin. Overlying the occipital bone, the underlying tissue was prepared in such a way that the atlanto-occipital membrane in between the occipital bone and the upper cervical vertebra was exposed. The remaining tissue above the atlanto-occipital membrane was cut off with a scalp incision until the atlanto-occipital membrane was clearly visible. The subsequent procedure was performed in the same manner. The total volume was approximately 80 to 120 μ l in rats and guinea pigs, and 100 to 250 μ l in rabbits respectively. The success rate was 90%. Failure etiologies were mainly related to dry aspiration, blood contamination of CSF and obstruction of the needle.

IMPACT OF KETAMINE/XYLAZINE ANESTHESIA ON ECG PARAMETERS, AUTONOMIC NERVOUS SYSTEM ACTIVITY, ACID-BASE BALANCE AND ION CONCENTRATION IN WISTAR RATS. CHRONOBIOLOGICAL VIEW

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Administration of anesthesia may influence specific aspects of *in vivo* animal experiments and is an especially important consideration for experiments conducted during the daytime. Although chronobiological

studies investigating interactions between general anesthesia and circadian rhythms are sparse, all suggest that general anesthetic agents have a significant effect on circadian rhythms. The aim of study was to assess the suitability of ketamine/xylazine anesthesia in chronobiological studies. The present study was performed using ketamine/xylazine-anesthetized (100 mg/kg/15 mg/kg ip.) female Wistar rats after adaptation to a light-dark (LD) cycle (12 h:12 h). Heart rate, rectal temperature, electrocardiographic parameters, autonomic nervous system activity, acid-base balance, and plasma concentrations of Na^+ , K^+ , Ca^{2+} and Cl^- were evaluated for their dependence on the LD cycle. LD differences were found in heart rate and rectal temperature, measured before and after administration of the anesthetic agent, in all electrophysiological parameters (except QT intervals), in amplitude P wave (except amplitude R and T waves) and in all parameters of heart rate variability (power HF, LF and VLF). LD differences in acid-base balance were found only for pO_2 and for HCO_3^- (except pH, pCO_2 , TCO_2 , BE, stHCO_3^- , O_2 saturation). LD differences in ion concentrations were found for Na^+ , Ca^{2+} and for Cl^- , not for K^+ . Results of the present study suggest that ketamine/xylazine anesthesia may be suitable for chronobiological studies. However, it is not appropriate for cardiovascular research because suppress significantly heart activity. During ketamine/xylazine anesthesia, parasympathetic tone predominates and sympathetic activity is depressed. Spontaneously breathing rats under ketamine/xylazine anesthesia are in an asphyxial state independent of the LD cycle in *in vivo* experiments.

DYSLIPIDEMIC DIET INFLUENCE ON BONE METABOLISM IN MALE RATS PHHP

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We investigated influence diet, whose composition influenced the lipide and bone metabolism and subsequent resistance to bone fracture at Prague hereditary hypercholesterolemic rats (PHHP). PHHP strain originated from Wistar rats with inbred crossing. Male rats PHHP were divided into 4 groups (6 rats in each of them). Group 1: (SD) fed standard laboratory diet; Group 2: (CHOL) standard lab diet enriched with cholesterol by 4%; Group 3: (MCDD) standard lab diet without choline and methionine, but supplemented with arginine; Group 4: (OA) standard lab diet enriched with 1% orotic acid. Rats were sacrificed at 28 days under anesthesia by exsanguination from the abdominal aorta. After *post mortem* bone mineral density was measured – bone mineral density (BMD, g/cm^2) of dual-energy absorption spectrophotometry (DXA, Hologic, Waltham, MA, USA). Subsequently they were excluded from the rat tail vertebrae and femurs. Femurs were broken in a controlled test device (Martin Kosek & Pavel Trnecka, Hradec Kralove, CZ). The serum leptin concentrations were determined (pg/ml), glucose (mmol/l) and insulin ($\mu\text{g}/\text{ml}$). Statistical analysis was performed using the software "SigmaStat 3.1" Jandel Scientific, San Rafael, CA, USA. Data are expressed as mean \pm standard error or as median (and percentiles of 25-75%). Serum leptin and insulin were unchanged. Serum glucose is lower in OA groups (7.6 \pm 0.2) vs SD (8.1 \pm 0.2; $p < 0.089$). Serum cholesterol changes were significant in all groups (SD 3.32 \pm 0.09; CHOL 6.95 \pm 0.70; MCDD 2.44 \pm 0.05; OA 1.14 \pm 0.07). Higher LDL CHOL values were in group CHOL (3.38 \pm 0.67 vs SD (0.34 \pm 0.04; $p < 0.002$). Insignificantly lower HDL was in CHOL (0.63 \pm 0.07) vs SD (1.69 \pm 0.07). Bone mineral density decreased in groups CHOL (0.137 \pm 0.002) and MCDD (0.137 \pm 0.007), whereas higher in OA (0.149 \pm 0.002) vs control SD (0.146 \pm 0.008). Similar trends are in the biomechanical resistance of the femurs. Three-point breaking: SD 135 \pm 5; CHOL 120 \pm 3; MCDD 129 \pm 5; OA 135 \pm 5. The necessary forces to fracture of the femoral neck: SD 137 \pm 10, CHOL 133 \pm 4; MCDD 116 \pm 7; OA 143 \pm 6. In conclusions, our results suggest that a diet enriched with cholesterol or diet without choline and methionine leads to a decrease in bone mineral density with force reduction for breaking femurs and their necks in rats PHHP. Leptin, insulin and blood glucose in this experiment did not seem significant.

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