FOLIA VETERINARIA

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

ISSN 0015-5748

4

XLVII • 2003

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, 47, 4, 2003

CONTENTS

FOLIA VETERINARIA, 47, 4: 173—176, 2003

ADRENERGIC INNERVATION OF THE SPLEEN IN RABBITS

Siroťáková, M., Schmidtová, K.*, Kočišová, M.*

Department of Experimental Medicine, Faculty of Medicine, P. J. Šafárik University
Tr. SNP 1, 040 11 Košice
***Department of Anatomy, Faculty of Medicine, P. J. Šafárik University**
Šrobárova 2, 040 01 Košice
The Slovak Republic

anatomia@uvm.sk

ABSTRACT

The innervation of the spleen in rabbits was studied. Adrenergic nerve components were visualized by the glyoxylic acid histofluorescence method. The adrenergic nerve components enter the spleen in a common bundle with arteries. In the organ itself they form characteristic dense periarterial plexiform arrangements of thicker and thinner nerve profiles, which are especially conspicuous around the *aa. centrales* running through the white pulp. The nerve fibres extend away from these plexuses into adjacent layers of fibrous trabeculae, further into the marginal layers of the periarterial lymphatic sheat (PALS) as well as into the mantle zone of the follicles. Several fine periarterial and solitary nerve profiles can be seen in the marginal sinuses and cords of the red pulp. In the fibrous capsule of the organ several specifically fluorescent adrenergic nerve fibres can also be seen which have an evident connection with the trabecular and parenchymal nerves of the spleen. Microscopic findings support the notion that adrenergic nerve components participate in the regulation of vascular motility as well as in the regulation of the micro-environment of the organ's own parenchyma.

Key words: adrenergic innervation; rabbits; spleen

INTRODUCTION

From the great number of published data on the nerve supply of the various compartments of the primary and secondary lymphoid organs (2, 5, 6, 11, 13), in various mammals it is evident that innervation of the spleen is not the object of only marginal interest. The central nervous system influences, via hormones and the autonomic nervous system, the functioning of the lymphoid organs. The presence of autonomic nerve fibres in the parenchyma of lymphoid organs establishes an anatomical link between the brain and the immune system to translate central neural processes into chemical signals that can influence specific functions of the immune system (2, 3, 4, 7, 8).

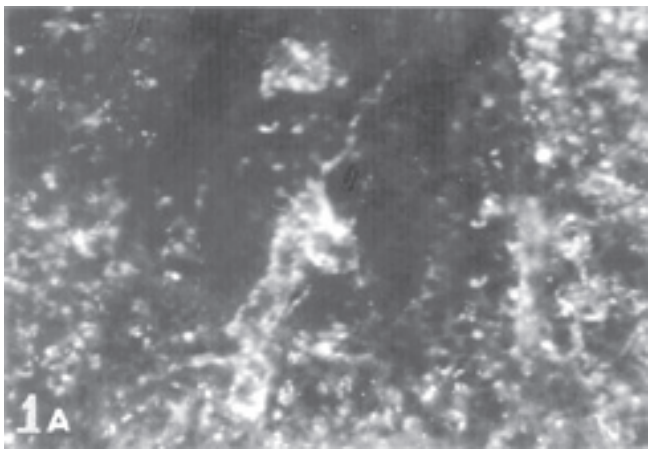
Results obtained by other authors (3, 4, 9, 12) indicate certain species-specificities of the innervation of the spleen in some mammals. In the present study, we have investigated adrenergic innervation of the spleen in rabbits as another laboratory animal.

MATERIAL AND METHODS

Clinical healthy adult animals of both sexes were used in the study. The spleen of twenty-five rabbits (Chinchilla 2,5—3,5 kg body weight) were examined. All animals were anaesthetized with pentobarbital (40 mg.kg^{-1} i.p.). Multiple tissue specimens were continuously collected from the spleen. The adrenergic nerve components in the spleen were visualized by means of the glyoxilic acid histofluorescence method (10). Cryostat sections (15–20 µm) were incubated in the 2 % glyoxylic acid incubation medium, than dried with a hair drier and kept at 100 °C. Individual sections were mounted in a nonfluorescent medium. Both microscopic examination and photographic documentation were performed using a Jenalumar 2 microscope.

RESULTS

Adrenergic nerve components enter the organ in a common bundle with *arteria lienalis* and its branches. In the organ itself, they border all its branches in the form of carrier nerve plexuses and also as more delicate plexuses consisting mainly of the pre-terminal and



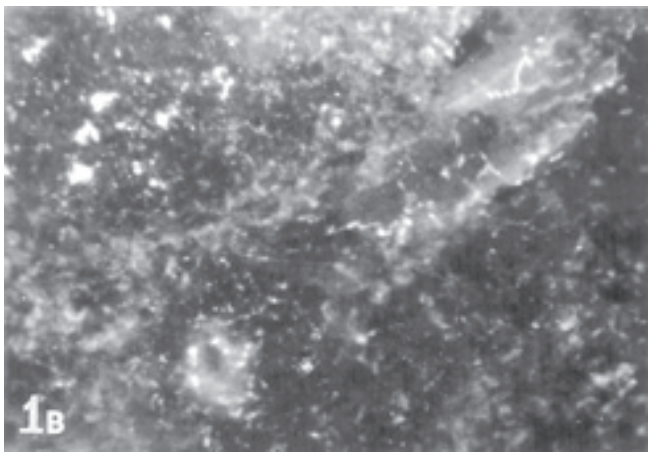


Fig. 1 A, B. The terminal parts of the arteriolar branches usually represented only one or two nerve fibres. Magn. × 250

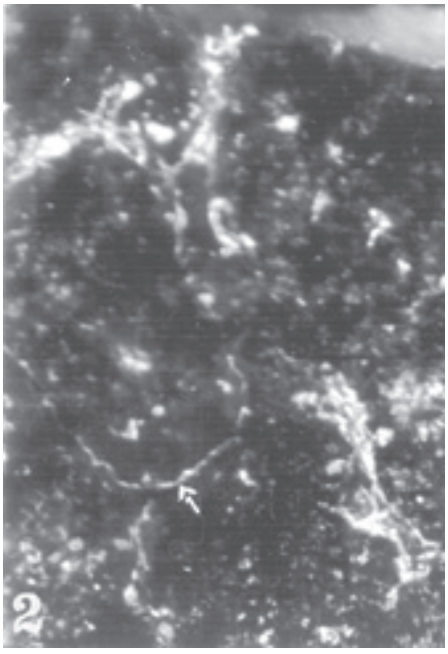


Fig. 2. Intrasplenic nerve components lying in the subcapsular parts of the parenchyma. Solitary nerve fibres in the red pulp are very rare (arrow). Magn. × 250

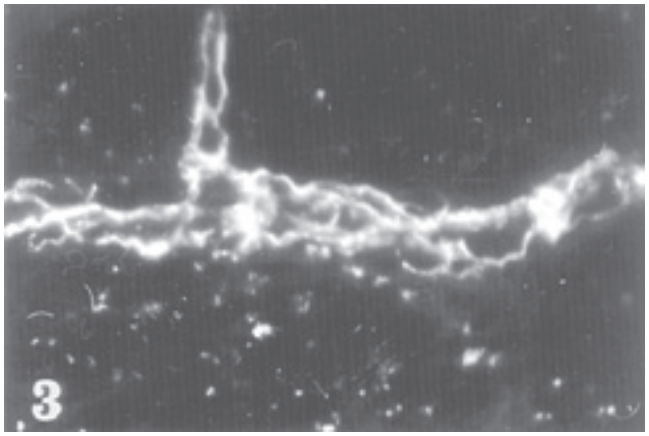


Fig. 3. Intensively fluorescent nerve fibres around the wall of the septum running arteriole and diversion *a. centralis*. Magn. × 450

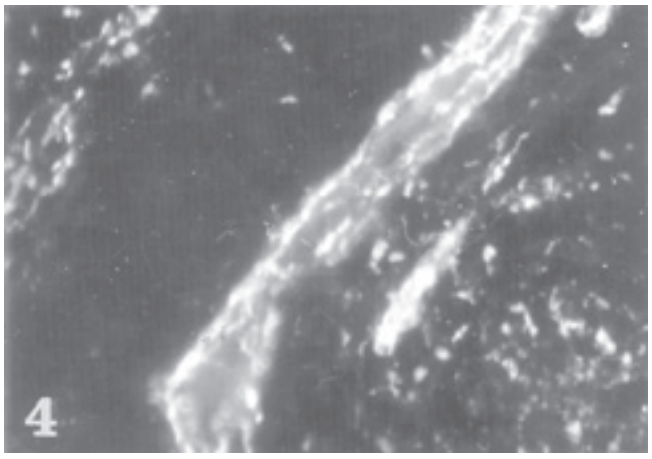


Fig. 4. Relatively rich periarteriolar nerve plexuses border the wall of *a. centralis* runs in the marginal parts of the fibrous septum. Magn. × 450

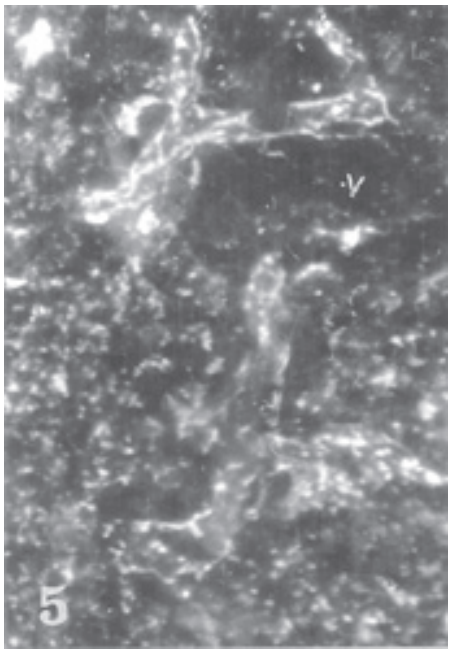


Fig. 5. Fine adventitial adrenergic nerve plexuses can be sporadically seen in the red pulp. Small venous sporadically twing do not have their own innervation (V). Magn. × 250

terminal varicose nerve fibres. These fine plexuses lie in close contact with the external side of the muscular media layer and are sometimes called "adventitial plexuses". The main perivascular nerve profiles were distributed in the fibrous trabeculae as well as in the periarteriolar lymphatic sheath (PALS). Some specifically fluorescent nerve fibres also pass through the trabeculae and capsula of the organ without apparent connection to the vascular branches. In very close proximity to the terminal branches of the central arterioles the accompanying nerve components usually represent only one or two nerve profiles (Figs. 1A, B).

Smaller nerve plexuses were found also in association with the vascular system, fibrous trabeculae and capsule. In the fibrous trabeculae, thicker nerve profiles run more less parallel with the appropriate arteries or arterioles, largely as intensively fluorescent isolated punctate or linear nerve profiles.

Nerve fibres associated with the capsula fibrosa were moderately dense, and travelled in or just beneath the capsula. From the capsular plexuses, some adrenergic nerve fibres, extend into the surrounding parenchyma, especially into the white pulp, near the capsula (Fig. 2).

Relatively, the greatest density of adrenergic nerve profiles within the spleen were around the central artery and its branches in the white pulp and the venous system (Fig. 3). From these vascular plexuses numerous fine nerve fibres radiate into the surrounding PALS and in the vicinity of the white pulp, with additional distribution along the inner region of the marginal zones (Fig. 4).

Within PALS they may be seen most often in the marginal zone between the red and white pulp. Adrenergic nerve fibres were largely confined to the parafollicular regions of the white pulp. The occurrence of specifically fluorescent adrenergic nerve fibres in the mantle zone of the lymphatic follicles were evident. Nerve fibres do not penetrate into the germinal centers of follicles. In the marginal sinuses and cords of the red pulp fine nerve fibres were also observed, but only rarely (Fig. 5).

A further conspicuous component of the microscopic findings of adrenergic innervation of the rabbit's spleen was represented by fine solitary nerve fibres deflecting away from the carrying periarterial nerve plexuses and also from the nerve components running through the trabeculae. The wall of large and larger splenic veins running through the trabeculae was partially and modestly innervated.

It is remarkable that intensive specifically fluorescent periarteriolar and solitary nerve profiles were a relatively abundant finding, also in the capsula *fibrosa lienalis*. Likewise in guinea pigs also, the direct connection between nerve components of the capsula with intralienal neural structures was often observed.

DISCUSSION

Our findings of adrenergic innervation of the rabbit spleen are in agreement with the data found in rats (1, 5, 4) that these nerves enter the organ together with arteries round which they form very conspicuous and typical thicker nerve arrangements that fulfil the role of a common carrying substrate. The vasomotor regulatory functions fulfil the fine nerve plexuses consisting of pre-terminal and terminal varicose fibres lying in close contact with the external side of the vascular media (2). Besides, in the diffusibility zone of the neurotransmitter released from these nerve formations, there are also other structural components of the organ, i.e. smooth muscular cells of vessel media, lymphatic vessels as well as fibrous trabeculae themselves.

It is well know that the spleen also plays an important role as a blood reservoir. Thus from the morphological aspect, the appropriate vasomotor functions are combined with reactions of smooth muscular cells in the fibrous trabeculae and capsula of the organ, appearing to be a factor that participates not only in the regulation of the blood flow through the spleen but in its accumulative functions (1).

The blood filtration, together with the primary immune responses on antigens, is supplied by blood into the spleen from the internal enviroment of the organism. So the micro-environment of the terminal branches of arterioles lying in the spleen are influenced by the neurotransmitter released from neural components as are the functions that depend on the relatively complicated microcirculation of the white and red pulp of the spleen.

In rabbits, similarly as in other rodents, adrenergic nerve components are found in the trabeculae and in close proximity to the vascular media of arterial branches and central arterioles, in the region of the T-dependent topography, but not in the B-dependent compartment.

It is known that lymphocyte functions are altered following noradrenaline (NA) depletion of the spleen and that NA plays a modulatory role in immune response and that sympathetic terminals may act with other accessory cells and further that this neurotransmitter may also influence individual cellular function such as proliferation, differentiation, expression of specific receptors, as well as synthesis and secretion of cellular products (8).

There are data (2) that the released neurotransmitter may also influence the collective interactions of cells of the immune system, such as primary and secondary antibody responses, cytotoxic T-lymphocyte activity and delayed-type hypersensitivity responses and so on (9).

The subsequent activation of T-lymphocytes could be influenced mainly within the range of PALS bordeline layer, whereas the activation of B-lymphocytes takes place mainly in PALS perifollicular and only partially in the marginal zone, the neurotransmitter could also influence the entry of lymphocytes into the thin-wall initial compartments of the venous bed (7, 8).

Our findings of adrenergic nerve fibres in the red pulp are in contrast with the data of other authors (3, 4, 8, 5) who have suggested that the spleen in the mouse or rat is richly innervated by sympathetic nerve fibres, but these nerve fibres do not enter the red pulp, because they extend only to the border of the white and red pulp.

From comparative morphological study, it can be stated that the patterns of adrenergic innervation of the spleen in rabbits agree in principle with those in rats and guinea pigs. Only the total number of nerve profiles supplying larger venous branches, or fibrous trabeculae, and their presence in the perifollicular topography, is in the guinea pig apparently higher than in rats and rabbits.

Our findings are in agreement with the data of other authors (2, 4, 8); that adrenergic nerve components supply not only the vasculature, but also the parenchymal components of the spleen and thus they can participate to a great extent, in the regulation of the immune process in this organ.

As is known, adrenergic innervation of the spleen represents only one of the means by which the CNS can communicate with the immune system, because the immune functions of the organ may also influence other systems, i. e. the neuroendocrine or peptidergic systems.

This work was supported by GRANT of LF UPJŠ No. 19/2002/IG4.

REFERENCES

1. Bellinger, D. L., Felten, S. Y., Collier, T. J., Felten, D. L., 1987: Noradrenergic sympathetic innervation of the spleen. IV. Morphometric analysis in adult and aged F344 rats. *J. Neurosci Res.,* 18, 55—63.

2. Felten, D. L., 1993: Direct innervation of lymphoid organs: Substrate for neurotransmitter signaling cells of the immune system. *Neuropsychobiol.,* 28, 110—112.

3. Felten, D. L., Felten, S. Y., Carlson, S. L., Olschowka, J. A., Livnat, S., 1985: Noradrenergic and peptidergic innervation of lymphoid tissue. *J. Immunol.,* 135, 755—765.

4. Felten, D. L., Ackerman, K. D., Wiegand, S. J., Felten, S. Y., 1987: Noradrenergic sympathetic innervation of the spleen: Nerve fibres associate with lymphocytes and macrophages in specific compartments of the splenic white pulp. *J. Neurosci.,* 18, 28—36.

5. Felten, S. Y., Olschowka, J. A., 1987: Noradrenergic sympathetic innervation of the spleen. II. Tyrosine hydroxylase (TH)-positive nerve terminals form synaptic-like contacts in the splenic white pulp. *J. Neurosci. Res.,* 18, 37—48.

6. Kočišová, M., Siroťáková, M., Schmidtová, K., 2003: NADPH-d positive innervation of the thymus in quails (In Slovak). *6th Morphological Day, Collection of the Reviews.* Košice, 61—62.

7. Madden, K. S., Felten, S. Y., Felten, D. L., Sundaresan, P. R., Livnat, S., 1989: Sympathetic neural modulation of the immune system. *Brain Behav. Immunol.,* 3, 72—81.

8. Madden, K. S., Moynihan, J. A., Brenner, G. J., Felten, S. Y., Felten, D. L., Livnat, S., 1994: Sympathetic nervous system modulation of the immune system. III. Alteration in T and B cell proliferation and differentiation *in vitro* following chemical sympathectomy. *J. Immunol.,* 49, 77—87.

9. Pellas, T. C., Weis, L., 1990: Deep splenic lymphatic vessels in the mouse: A route of splenic exit for recirculating lymphocytes. *Am. J. Anat.,* 187, 347—354.

10. Shvalev, V. N., Zhuckova, N. I., 1987: An improvement in histochemical findings in adrenergic nerve elements in glyoxylic acid solution with the acid of dimethylsulphoxide (DMSO) (In Russian). *Arkh. Anat.,* 93, 91—91.

11. Schmidtová, K., 2003: Cholinergic innervation of the spleen in rat (In Slovak). *6th Morphological Day, Collection of the Reviews.* Košice, 124—125.

12. Siroťáková, M., Maretta, M., Marettová, E., 2001: Butyrylcholinesterase positive innervation of the spleen in rabbits (In Slovak). *New Trends in Morphology Collection of Scientific Works,* Martin, 184—185.

13. Stopek, D., Siroťáková, M., Kočišová, M., 1996: Adrenergic nerve components of the enteral nervous system in the appendix of adult rabbits. A fluorescent microscopic study. *37th Meeting of Czech-Slovak Anat. Society,* Brno, Abstract, 27.

Received September 3, 2003

FOLIA VETERINARIA, 47, 4: 177—182, 2003

THE RADIOPROTECTIVE EFFECT OF THE BACTERIAL EXTRACT BRONCHO-VAXOM ON HISTONES IN REGENERATING AND NORMAL RAT LIVER

Kožurková, M., Mišúrová, E., Kropáčová, K.

Institute of Biological and Ecological Sciences, P. J. Šafárik University
Moyzesova 11, 041 54 Košice
The Slovak Republic

kozurkova@kosice.upjs.sk

ABSTRACT

We have studied the influence of the immunomodulator Broncho-Vaxom (Biogal Pharmaceutical Works, Debrecen, Hungary, under licence from OM Laboratories, Geneva, Switzerland) on the concentration and total content of histones and proportion of individual histone fractions in the normal and regenerating liver of rats irradiated with doses of 3, 6 or 9 Gy of gamma radiation. Animals in all groups were partially hepatectomized (seventy per cent of liver mass removed) within thirty minutes or six and thirteen days after irradiation and they were examined thirty hours after resection, i.e., on the first, seventh and fourteenth days after irradiation. Broncho-Vaxom was administered intraperitoneally at a dose of 1.5 mg/rat twenty-four hours before irradiation. We have found that the administration of Broncho-Vaxom diminished the loss of histones induced by radiation in both normal and regenerating livers on the first day after radiation. A beneficial effect was found also on the seventh day but only in the normal liver. In the regenerating liver, the effect of Broncho-Vaxom was less noticeable than in the normal liver.

Key words: Broncho-Vaxom; gamma radiation; histones; liver; rat

INTRODUCTION

Radiotherapy is the most common modality for treating human cancers. Eighty percent of cancer patients need radiotherapy at some time or other, for curative or palliative purpose. To obtain optimum results, a judicious balance between the total dose of radiotherapy delivered and threshold limit of the surrounding normal critical tissues is required. In order to obtain better tumour control using a higher dose, normal tissues should be protected against radiation injury. Thus, the role of radioprotective compounds is very important in clinical radiotherapy (27).

Ionizing irradiation causes damage to living tissues through a series of molecular events. Because human tissues contain about eighty per cent water, the major radiation damage is due to the free radicals, generated by the action of radiation on water. The major free radicals resulting from aqueous radiolysis include $OH, H, HO_2^{\cdot}, H_3O^+$, etc. (6, 33, 34). The free radicals react with cellular macromolecules such as DNA, RNA, proteins etc. and cause cell dysfunction and mortality. The radiation damage to cells is potentiated or mitigated depending on several factors including the presence of oxygen. Oxidative damage to the genetic material, i.e. DNA, plays a major role in mutagenesis and carcinogenesis. (12).

DNA, in complex with histones and non-histone proteins is assembled in a highly ordered chromatin structure. The core of nucleosomes, the basic subunits of eukaryotic chromatin, is an octamer composed of two molecules of histone fractions H2A, H2B, H3 and H4 and a stretch of 146 bp of DNA (24, 38). Histone fraction H1 is associated with linker DNA and links the adjacent nucleosomes. It acts as a general repressor at one level of the regulation of gene expression by organising nucleosomes into a condensed form of chromatin, thereby making the DNA inaccessible to transcription machinery (5, 10, 29).

Ionizing irradiation causes profound changes in histones and nucleic acids in the proliferating and quiescent tissue (18, 19, 36, 37). The radiation changes can be prevented by administration of some radioprotective agents. Radioprotective compounds can be classified as radioprotectants, adaptogens and absorbents. Radioprotectants include: sulphhydryl compounds, antioxidants, ACE inhibitors, DNA binding ligands, immunomodulators (lipopolysacharides, prostaglandins, plant extracts) and other compounds.

Immunomodulator Broncho-Vaxom® (BV) is a lyophilized alkalic bacterial extract, which is used as a poly-valent immunotherapeutic agent especially in the treatment of respiratory tract infection (15, 30). It acts also as a stimulator of radioresistance. Therefore BV is also included among the adaptogens. Adaptogens are natural protectors, which offer chemical protection against low levels of ionizing radiation. They are generally extracted from the cells of plants and animals and have only minimal toxicity. Adaptogens can influence the regulatory systems of exposed organisms, mobilize the endogenous background of radioresistance and immunity, and in this way intensify the overall non-specific resistance of an organism. The action mechanism of BV is not yet fully understood. Experimental studies indicate that it enhances immune responses, both cellular (4, 7) and humoral immune responses (2, 7).

Fedoročko *et al.* (8) have found a radioprotective effect of Broncho-Vaxom on haemopoiesis in mice. The administration of BV twenty-four hours before whole body gamma irradiation accelerated the recovery of haemopoietic stem cells in the bone marrow (CFU-S colony forming units in spleen, GM-CFC-granulocyte-macrophage colony forming cells) and cell numbers in the peripheral blood (23). In the regenerating rat liver, administration of this agent resulted in an alleviation of latent radiation injury, which was indicated by relative increase in mitotic index, decrease in chromosome aberrations (20) and partially by mitigation of RNA and DNA changes (13).

In the present paper, we have investigated whether administration of Broncho-Vaxom modifies the radiation-induced changes in other part of chromatin, histones, in the normal and regenerating liver of rats and thus we have tried to contribute to the understanding of the effect of this immunomodulating preparation.

MATERIAL AND METHODS

Experiments were performed on male Wistar rats (SPF), aged twelve weeks and weighing 290 ± 20 grams at the beginning of the experiment. Animals were kept under standard conditions (temperature 22—24 °C, natural light rhythm), fed and watered *ad libitum.* They were housed in cages, with five to six animals in each.

Research was conducted according to the principles enunciated in the *"Guide for Care and Use of Laboratory Animals"*, prepared by the State Veterinary Administration of the Slovak Republic Bratislava.

Experimental rats were divided into seven groups according to the application of the tested substance and radiation dose. Analyses were performed on five to six animals from each group at three time intervals, on the first, seventh and fourteenth day after irradiation:

Group 1: C-non-treated, control rats

Group 2: BV-application of Broncho-Vaxom (1.5 mg/rat)

Groups 3—5: I-3 Gy, I-6 Gy, I-9 Gy – irradiation with a dose of 3 Gy, 6 Gy or 9 Gy, respectivelly

Groups 6—8: BV + I-3, BV + I-6 Gy, BV + I-9 Gy – application of Broncho-Vaxom (1.5 mg/rat) + irradiation with a dose of 3, 6 or 9 Gy, respectively Broncho-Vaxom (Biogal Pharmaceutical Works, Debrecen, Hungary, under licence from OM Laboratories, Geneva, Switzerland) is composed of lyofilized fractions of the eight most common bacteria of the upper respiratory tract (*Haemophilus influenzae, Diplococcus pneumoniae, Klebsiella pneumoniae, Klebsiella ozaenae, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans, Neisseria catarrhalis*). The preparation is free of endotoxins (less than 0.0002 % by the *Limulus* and pyrogenicity test) (3). Just before use, the drug was re-suspended in PBS (pH 7.4) in a volume 1.2 ml (1.5 mg) per rat and administered intraperitoneally. Control animals received 1.2 ml PBS at the same time.

Rats were irradiated with a single whole body dose of 3, 6 or 9 Gy by gamma rays from ^{60}Co source (apparatus Chisostat, the Czech Republic) with a dose rate of 348 mGy/min, 24 h after Broncho-Vaxom injection.

Rats in all groups were 2/3 hepatectomized within thirty minutes after irradiation or on the sixth and thirteenth day after irradiation. Partial hepatectomy was performed under light ether anaesthesia, in the morning hours (8.00—10.00 a.m.) according to the standard method (16). There was no operative and postoperative mortality with the exception of groups of non-protected as well as protected rats irradiated with the dose of 9 Gy, where no animal survived operation on the fourteenth day.

Isolation of nuclei and histone extraction was done according to the method of Grünicke *et al. (12).*

Protein concentration was determined spectrophotometrically (Hitachi, Tokyo, Japan) by the method of Lowry *et al.* (22) using bovine serum albumin as a standard.

Electrophoresis was carried out using the method of Panyim and Chalkley (31).

Histones were stained with amido black B. The relative proportion of histone fractions was determined spectrophotometrically on densitometer Shimadzu CS-930 (Japan).

The experimental data were statistically evaluated by Peritz' F-test (14). They are given as mean ± S.E.M. on the figures and tables.

RESULTS

Normal liver. Administration of Broncho-Vaxom alone did not result in any significant changes of histone concentration and content in the normal liver of rats with the exception of decrease in histone content on the fourteenth day (Figs. 1A, B).

Irradiation with lower doses caused a decrease in concentration of histones on the first day and mainly on the fourteenth day after irradiation. Because of the concomitant increase in liver weight, the decrease in concentration was not accompanied by an adequate decrease in the total content of histones. The total content of histones decreased only on the first and seventh day after irradiation with the dose of 9 Gy.

Administration of Broncho-Vaxom to animals irradiated with all doses of irradiation alleviated the decrease in histone concentration and content in comparison with non-protected irradiated animals at earlier intervals of investigation.

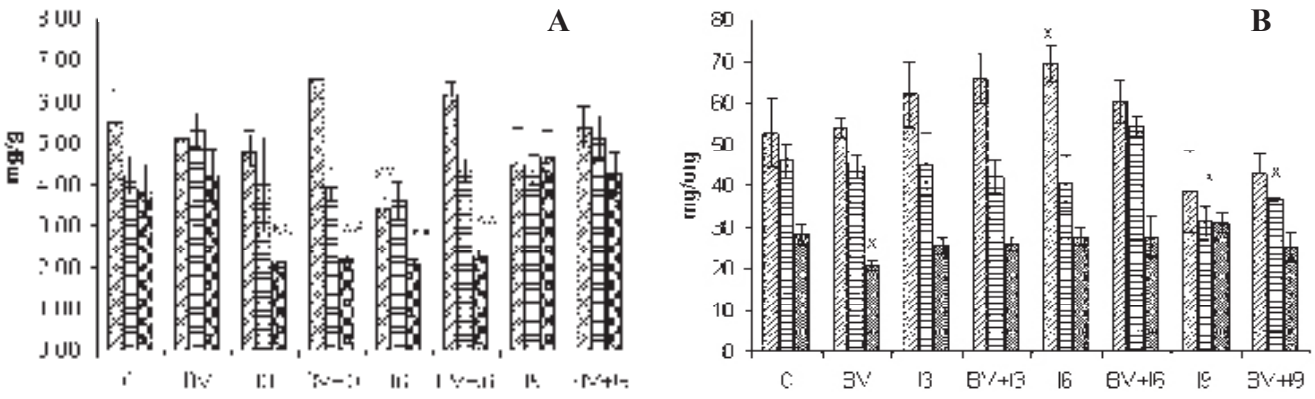


Fig. 1. Concentration (A) and content (B) of histones in the normal liver of rats after application of Broncho-Vaxom (1.5 mg/rat) and/or gamma radiation (3, 6 or 9 Gy)

Group 1: C-non-treated, control rats
Group 2: BV-application of Broncho-Vaxom (1.5 mg/rat)
Groups 3—5: I-3 Gy, I-6 Gy, I-9 Gy — irradiation with the dose of 3 Gy, 6 Gy or 9 Gy, respectivelly
Groups 6—8: BV + I-3, BV + I-6 Gy, BV + I-9 Gy — application of Broncho-Vaxom (1.5 mg/rat) + irradiation with the dose of 3, 6 or 9 Gy, respectivelly

x — P≤0.05, xx — P≤0.01: compared with control group
o — P≤0.05, oo — P≤0.01: compared with the corresponding irradiated groups

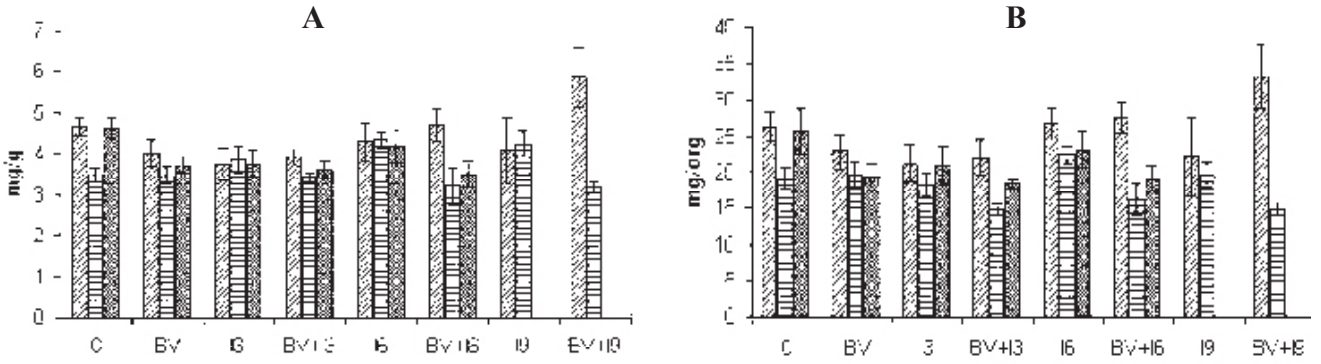


Fig 2. Concentration (A) and content (B) of histones in the regenerating liver of rats after application of Broncho-Vaxom (1.5 mg/rat) and/or gamma radiation (3, 6 or 9 Gy)

Group 1: C-non-treated, control rats
Group 2: BV-application of Broncho-Vaxom (1.5 mg/rat)
Groups 3—5: I-3 Gy, I-6 Gy, I-9 Gy — irradiation with the dose of 3 Gy, 6 Gy or 9 Gy, respectivelly
Groups 6—8: BV + I-3, BV + I-6 Gy, BV + I-9 Gy — application of Broncho-Vaxom (1.5 mg/rat) + irradiation with the dose of 3, 6 or 9 Gy, respectivelly

x — P ≤ 0.05, xx — P≤0.01 compared with control group
o — P ≤ 0.05, oo — P≤ 0.01 compared with the corresponding irradiated groups

In the relative proportion of individual histone fraction, there were no differences observed between the groups of animals treated and non-treated with Broncho-Vaxom alone except an increase in the relative proportion of fraction H4 on the first day of examination (Table 1).

The total body irradiation caused the deep decrease of the fraction H1 after dose of 9 Gy on the first and fourteenth days.

Histone fractions H2A, H2B and H3 were not separated distinctly by gel electrophoresis, therefore we determined only one common value. Irradiation with all the doses caused a decrease in these values on the first day. On the following few days the relative proportion of these fractions was restored and on the fourteenth day a significant increase occurred after the doses of 6 and 9 Gy.

The opposite sequence of changes was found in fraction H4.

Application of Broncho-Vaxom twenty-four hours before irradiation alleviated most of the histone fraction changes.

In the H1 fraction, the relative proportion of histone subfraction $H1^0$ was increased in all experimental groups, maximally in groups of non-protected as well as protected rats irradiated with the highest dose, on the first day. On the seventh day the proportion of this subfraction was increased only in the groups of rats irradiated with the doses of 6 Gy and especially of 9 Gy; until the

Table 1. The relative portion of the histone fraction (%) in the normal liver of rats first, seventh and fourteenthth days after the application of Broncho/Vaxom (1.5 mg/rat) and/or gamma radiation (3, 6 or 9 Gy)

Groups/ Time	1st day				7th days				14th days			
C	H1	H2+H3	H4	H1°	H1	H2+H3	H4	H1°	H1	H2+H3	H4	H1°
BV	31.2 ± 1.5	55.7 ± 1.1	13.1 ± 0.8	13.8 ± 0.5	28.4 ± 2.5	57.1 ± 2.1	14.5 ± 0.6	18.6 ± 3.9	32.3 ± 1.0	53.8 ± 0.9	13.8 ± 0.4	18.7 ± 2.4
I-3	27.3 ± 1.7	55.2 ± 0.7	18.2** ± 2.0	22.2** ± 3.0	28.9 ± 0.9	54.3 ± 1.4	19.1 ± 3.4	18.3 ± 1.4	32.8 ± 2.6	52.2 ±1.8	14.9 ± 1.0	19.5 ± 1.3
BV+I-3	31.5 ± 0.1	52.2* ± 0.4	16.2* ± 1.1	18.5** ± 0.7	26.3 ± 1.8	60.0 ± 2.2	13.6 ± 0.9	16.2 ± 2.2	30.0 ± 0.6	54.1 ± 0.4	15.9 ± 0.2	15.1 ± 1.9
I-6	30.1 ± 0.1	52.3* ± 1.0	17.5** ± 0.9	23.8** ± 2.8°°	31.7° ± 2.7	55.3° ± 1.5	12.9 ± 1.2	20.7 ± 4.6	33.8° ± 0.4	52.1 ± 0.6	14.0 ± 0.4	21.0°° ± 1.8
BV+I-6	32.4 ± 0.9	53.4 ± 0.8	14.2 ± 1.3	24.2** ± 4.5	30.5 ± 3.1	54.6 ± 2.4	14.7 ± 0.5	23.9* ± 1.3	29.9 ± 2.3	58.7** ± 0.8	13.6 ± 0.7	17.0 ± 3.1
I-9	31.2 ± 0.1	52.9* ± 0.1	15.3 ± 0.6	23.8** ± 2.8	30.1 ± 0.9	56.3 ± 1.7	13.4 ± 1.1	16.2°° ± 2.2	30.8 ± 0.6	54.3° ± 0.4	14.8 ± 0.4	10.5** ± 1.7°
BV+I-9	19.1** ± 2.1	51.4* ± 5.2	17.2** ± 1.8	37.8** ± 3.7	33.6* ± 1.5	58.8 ± 0.8	7.7* ± 0.3	34.8** ± 0.7	25.2** ± 0.7	59.0** ± 0.3	15.8 ± 0.7	10.3** ± 1.2
	21.6** ± 1.0	65.0** ± 3.9°°	17.5 ± 2.2	38.0** ± 3.6	26.0°° ± 1.6	60.0 ± 0.4	14.4°° ± 0.6	21.9°° ± 1.2	25.7** ± 0.8	58.4** ± 0.3	15.9 ± 0.6	13.0** ± 1.5

Legend: C-non-treated control rats
BV-application of Broncho-Vaxom (1.5 mg/rat)
I-3 Gy, I-6 Gy, I-9 Gy — irradiation with the dose of 3 Gy, 6 Gy or 9 Gy, respectivelly
BV+I-3, BV+I-6 Gy, BV+I-9 Gy — application of Broncho-Vaxom (1.5 mg/rat) + irradiation with the dose of 3, 6 or 9 Gy, respectively
*—$P \leq 0.05$, **—$P \leq 0.01$ compared with control group
o—$P \leq 0.05$, oo—$P \leq 0.01$ compared with the corresponding irradiated groups

Table 2. The relative portion of the histone fraction (%) in the regenerating liver of rats first, seventh and fourteenth days after the application of Broncho/Vaxom (1.5 mg/rat) and/or gamma radiation (3, 6 or 9 Gy)

Groups/ Time	1st day				7th days				14th days			
	H1	H2+H3	H4	H1°	H1	H2+H3	H4	H1°	H1	H2+H3	H4	H1°
C	27.5 ± 3.4	58.2 ± 1.7	14.2 ± 1.5	14.1 ± 2.1	25.1 ± 3.1	59.0 ± 0.2	15.8 ± 1.1	16.6 ± 2.9	29.5 ± 1.3	59.6 ± 2.3	12.6 ± 2.4	20.0 ± 2.4
BV	23.9 ± 1.4	59.7 ± 1.4	16.6 ± 0.7	12.9 ± 1.5	28.9 ± 1.0	55.6* ± 2.3	16.7 ± 0.4	22.2 ± 5.3	28.3 ± 1.1	55.8 ± 1.3	16.8 ± 2.1	25.7* ± 1.3
I-3	21.5* ± 0.3	60.5* ± 0.8	17.9 ± 1.2	18.8 ± 1.1	29.5 ± 0.9	54.7* ± 0.8	15.7 ± 0.2	23.9* ± 2.7	26.9 ± 1.8	59.0 ± 4.0	14.1 ± 2.2	20.0 ± 4.9
BV + I-3	24.2*° ± 0.2	57.0° ± 1.1	14.4 ± 1.4	15.0 ± 3.6	32.1** ± 1.7	53.0* ± 1.3	14.8 ± 0.6	26.6** ± 4.6	30.5 ± 2.4	52.1 ± 4.6	17.4** ± 0.4	13.5** ±1.8°°
I-6	23.2 ± 1.0	61.0 ± 1.0	15.8 ± 1.6	16.3 ± 0.6	24.4 ± 4.1	58.2 ± 0.3	17.3 ± 3.2	15.2 ± 4.9	30.5 ± 1.9	54.4 ± 1.3	15.0 ± 1.5	21.4 ± 3.2
BV + I-6	29.0°° ± 0.1	63.4 ± 0.1	16.1 ± 0.6	20.1 ± 2.8	32.4** ± 0.9	54.3* ± 1.7	13.3°° ± 1.1	25.6* ± 2.2	28.9 ± 0.6	55.0 ± 0.4	16.0* ± 0.4	16.6* ± 1.7°
I-9	16.1** ± 0.3	65.3** ± 0.2	18.5** ± 0.3	40.1** ± 0.4	19.3* ± 1.2	64.2* ± 1.4	16.5 ± 0.2	30.4** ± 3.7				
BV + I-9	16.4** ± 1.8	67.9** ± 2.9	13.1 ± 2.6	38.1** ± 1.5	21.4 ± 1.2	61.6 ± 0.2	16.9 ± 0.8	22.3* ± 1.8				

Legend: See Table 1

fourteenth day the values diminished below the control level. Administration of Broncho-Vaxom resulted only in temporary alleviation of the H1° subfraction changes on the seventh day after irradiation with the dose of 6 Gy and 9 Gy.

Regenerating liver. Thirty hours after the partial hepatectomy, the concentration of histones in the regenerating liver was similar to a normal liver, however, due to the previous resection of seventy per cent of the liver mass, the total content of histones was still only about half of the preoperative value (Fig. 2A, B).

Administration of Broncho-Vaxom alone did not bring about significant histone changes in the regenerating liver over the whole investigated period. Irradiation

with all doses caused only mild and transient decrease in concentration or content of histones.

The application of immunomodulator twenty-four hours before irradiation manifested its beneficial effect on histones only in animals irradiated with the dose of 9 Gy on the first day. On the seventh day or possibly on the fourteenth day after irradiation with all doses, the changes of histones in BV-protected rats were even deeper than in non-protected ones.

In the regenerating liver, gamma irradiation with all doses caused a decrease in the relative proportion of histone fraction H1 on the first day after irradiation. Later on, the decrease in histone H1 was found only in rats irradiated with the highest dose of irradiation.

Administration of Broncho-Vaxom alleviated the changes in the H1 fraction induced by radiation mainly after the doses of 3 and 6 Gy. In other groups of irradiated rats and other time intervals, the administration of BV caused a transient increase in the relative proportion of this histone fraction (Table 2).

In relative proportions of H2 + H3 and H4 histone fractions only small changes were found.

The proportion of subfraction H1° in histone fraction H1 was increased in rats irradiated with all doses, mainly with the dose of 9 Gy, on the first and seventh day. Administration of BV alleviated the increase in relative proportion of the H1° subfraction after the highest dose of irradiation. In rats irradiated with the lower doses, BV pretreatment had an opposite effect at the same time.

DISCUSSION

In previous experiments, Fedoročko *et al.* (8, 9) have found an increased survival of mice irradiated with the lethal dose of gamma irradiation and protected by administration of Broncho-Vaxom twenty-four hours before irradiation. Administration of BV twenty-four hours before irradiation with lower doses of gamma radiation had a beneficial effect on haemopoiesis (8, 9) and on the radiation-induced changes of nucleic acids in rat tissues differing with regard to proliferative activity and radioresistance. The beneficial effect of the immunomodulator on the development and recovery of nucleic acids changes was demonstrated in haemopoietic tissues of bone marrow and spleen. In the regenerating liver, the effect of Broncho-Vaxom was less noticeable.

In this experiment, administration of Broncho-Vaxom twenty-four hours before irradiation with a dose of 9 Gy did not prevent the postoperative mortality of rats. Similarly, as in previous paper (13), our results showed that the beneficial effect of Broncho-Vaxom on histones in the regenerating liver was smaller compared to the normal liver.

It is interesting that the radioprotective effect of Broncho-Vaxom was small in the regenerating liver since, in general, this immunomodulator demonstrated a beneficial effect on proliferating cells and hepatocytes divided very rapidly at the time of investigation (thirty hours after partial hepatectomy).

The different effect of Broncho-Vaxom on the tissues investigated can be related to the fact that the great majority of hepatic cells were in the G_0 phase of the cell cycle at the time of BV administration and/or irradiation.

Broncho-Vaxom induces secretion of cytokines (IL-1 and prostaglandins), the radioprotective effect of which is known under both *in vitro* and *in vivo* conditions (28, 39). According to a number of authors (17, 26), cytokine IL-1b inhibits DNA synthesis in hepatocytes and, in such a way, gives them the time necessary for the repair of DNA damage. Synthesis of histones in the regenerating liver is coupled with DNA replication, i.e. it occurs mainly sixteen to twenty-four hours after partial hepatectomy (21). Ashami (1) has shown that synthesis of histone H1 slightly precedes DNA synthesis. These findings could contribute to the explanation of the less marked effect of Broncho-Vaxom on histones as opposed to nucleic acids.

The increase in the relative proportion of $H1^0$ histone subfraction after irradiation in the normal and regenerating liver is in accordance with our previous findings (18, 19). Subfraction $H1^0$ is typical for non-proliferating and terminally differentiated cells, including hepatocytes (25, 35) and its relative proportion in the histone H1 fraction is in relation to the degree of chromatin condensation (40). Therefore, an increase in the relative proportion of H1° histone subfraction in the normal and regenerating liver of irradiated rats suggested that a higher portion of hepatocytes could be recruited into a non-proliferating stage after irradiation and this is not reversed by administration of Broncho-Vaxom.

Acknowledgements

The authors greatly acknowledge Mrs. Oľga Staňová for her excellent assistence. This work was partially suported by a grant from the Ministry of Education and Science of the Slovak Republic No 1/9205/02.

REFERENCES

1. Ashami, K., 1978: Synthesis and phosphorylation of histone H1 and high mobility group protein in the regenerating rat liver after X irradiation. *Radiat. Res.,* 109, 216—226.

2. Bosch, A., Lucerna, F., Pares, R., Jofre, J., 1983: Bacterial immunostimulant (Broncho-Vaxom) versus Levamisole on the humoral immune response in mice. *Int. J. Immunopharmacol.,* 5, 107—116.

3. Botex, C., Cristau, B., Corazza, J. L., Mougin, B., Fontanges, R., 1988: Effects of two bacterial extracts, OM-89 and Broncho-Vaxom, on IL-1 release and metabolic activity of murine macrophage cell-line. *Int. J. Immunother.,* 4, 203—212.

4. Clot, J., Andary, M., 1980: Immunostimulation induite par un lysat bacterial lyophiliese. Etude *in vitro* des responses specifiques et non specifiques. *Med. Hug.* (Geneva), 38, 2776—2782.

5. Csordas, A., 1990: On the biological role of histone acetylation. *Biochem. J.,* 256, 23—38.

6. Dragaric, I. G., Dragaric, Z. D., 1971: *The Radiation Chemistry of Water.* Academic Press, New York, 256 pp.

7. Emmerich, B., Emslander, H. P., Pachman, K., Hallek, M., Milatovic, D., Bush, R., 1990: Local immunity in patients with chronic bronchitis and the effects of a bacterial extract, Broncho-Vaxom, on T-lymphocytes, macrophages, gamma interferon and secretory immunoglobulin A in bronchoalveolar lavage fluid and other variables. *Respiration,* 57, 90—99.

8. Fedoročko, P., Brezáni, P., Macková, O. N., 1992: Radioprotection of mice by the bacterial extract Broncho-Vaxom: haemopoietic stem cells and sutvival enhancement. *Int. J. Radiat. Biol.,* 61, 511—518.

9. Fedoročko, P., Macková, O. N., Brezáni, P., Kopka, M. 1994: Administration of the bacterial extract Broncho-Vaxom enhances radiation recovery and myolopoietic regeneration. *Immunopharmacology,* 28, 163—170.

10. Fensenfeld, G., 1992: Chromatin as an essential part of the transcriptional mechanism. *Nature,* 355, 219—224.

11. Fridovich, I., 1978: The biology of oxygen radicals. *Science,* 201, 875—880.

12. Grünicke, H. H., Yamada, I., Natsumeda, I., Helliger, W., Puschendorf, B., Weber, G., 1989: Histone acetyltransferase activity in rat hepatomas. *Cancer Res. Clin. Oncol.,* 115, 435—438.

13. Haková, H., Mišúrová, E., Kropáčová, K., 1997: Modification of postradiative changes of nucleic acids by bacterial extract Broncho-Vaxom in rat tissues. *Folia biologica* (Praha), 43, 231—237.

14. Harper, J., 1994: Peritz' F-test: basic program of a robust multiple comparison test for statistical analysis of all differences among group means. *Comp. Biol. Med.,* 14, 437—445.

15. Heinz, B., Schlenter, W., Kirsten, R., Nelson, K., 1989: Clinical efficacy of Broncho-Vaxom in adult patients with chronic purulent sinusitis – a multicentric, placebo-controled, double-blind study. *Int. J. Clin. Pharmacol. Ther. Toxicol.,* 27, 530—534.

16. Higgins, G., Anderson, R., 1931: Experimental pathology of the liver. Restoration of the liver of the white rat following partial surgical removal. *Arch. Pathol.,* 12, 187—202.

17. Koch, K. S., Lu, X. P., Brenner D. A., Fey G. H., Martinez-Conde, A., Lefert, H. L., 1990: Mitogens and hepatocyte growth control in vivo and in vitro. *In Vitro Cell. Dev. Biol.,* 26, 1011—1023.

18. Kožurková, M., Mišúrová, E., Kropáčová, K., 1994: Aging and radiation induced alteration in liver histones. *Neoplasma,* 41, 89—94.

19. Kožurková, M., Mišúrová, E., Kropáčová, K., 1995: Effect of aging and gamma radiation on acetylation of rat liver histones. *Mech. Ageing Dev.,* 78, 1—14.

20. Kropáčová, K., Mišúrová, E., 1999: Radioprotective effect of Broncho-Vaxom on the development of latent injury in rat liver. *Vet. Med.-Czech.,* 44, 279—287.

21. Kuehl, L., 1979: Synthesis of high mobility group proteins in regenerating rat liver. *J. Biol. Chem.,* 254, 7276—7281.

22. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J., 1951: Protein measurement with Folin phenol reagent. *J. Biol. Chem.,* 193, 265—275.

23. Macková, N. O., Fedoročko, P., 1993: Pre-irradiation haematological effects of the bacterial extract Broncho-Vaxom and postirradiation acceleration of recovery from radiation-induced haematopoietic depression. *Drugs Exp. Clin. Res.,* 19, 143—150.

24. McGhee, J. D., Nikolj, M., Fensenfeld, G., Rau, D. C., 1983: Higher order structure of chromatin orientation of nucleosomes with the 30 nm chromatin solenoid is independent of species and spacer lengh. *Cell,* 33, 831—836.

25. Medvedev, Zh. A., Medvedeva, M. N., 1990: Age-related changes of the histone H1 and H1° histone variants in murine tissues. *Exp. Gerontol.,* 25, 189—200.

26. Michalopoulos, G., 1990: Liver regeneration: molecular mechanisms of growth control. *FASEB J.,* 4, 176—184

27. Nair, C. K. K., Parida, D. K., Nomura, T., 2001: Radioprotectors in radiotherapy. *J. Radiat. Res.,* 42, 21—37.

28. Neta, R., Douches, S., Openheim, J. J., 1986: Interleukin 1 is a radioprotector. *J. Immunol.,* 136, 2483—2485.

29. Nillson, P., Manneranaa, R. M., Okarinen, J., Gondström, T., 1992: DNA binding of histone H1 is modulated by nucleotides. *FEBS Lett.,* 313, 67—70.

30. Palma-Carlos, A. G., Palma-Carlos, M. L., Inacio, F. F., Sousa Uva, A., 1987: Oral immunotherapy with lyophilized bacterial lysate in patients with recurrent respiratory tract infection. *Int. J. Immunother.,* 3,123—130.

31. Panyim, S., Chalkley, R., 1969: A new histone found in mammalian tissues with little cell division. *Biochem. Biophys. Res. Commun.,* 37, 1042—1047.

32. Patchen, M. L., Chirigos, M. A., Brook, I., 1988: Use of glukan-P and sixteen others immunopharmaceutical agents in prevention of acute radiation injury. *Comments Toxicol.,* 2, 217—231.

33. Pradhan, D. S., Nair, C. K. K., Sreenivasan, A., 1973: Radiation injury repair and sensitization of microorganisms. *Proc. Ind. Natl. Sci. Acad.,* 39B, 516—530.

34. Scholes, G., 1983: Radiation effects on DNA: The Silvanus Thomson memorial lecture, April 1982. *Br. J. Radiol.,* 56, 221—231.

35. Srebreva, L., Kachaunova, A., Zlatanova, J., 1991: The occurrence and properties of histone H1° in quiescent rabbit tissues. *Int. J. Biochem.,* 23, 189—194.

36. Süliová, J., Mišúrová, E., 1982: Effect of X-irradiation on histones and DNA in rat thymus. *Radiobiol. Radiother.,* 23, 667—674.

37. Süliová, J., Praslička, M., Mišúrová, E., 1983: Comparison of the efficiency of acute and continuous radiation on changes of histones and DNA in the thymus of rats. *Bratisl. lek. listy,* 80, 661—668.

38. VanHolde, K. E., Lohr, D. E., Robert, Ch., 1992: What happens to nucleosomes during transcription? *J. Biol. Chem.,* 267, 2837—2840.

39. Walden, T. L., Patchen, M. L., Snyder, S. L., 1987: 16,16-dimethyl prostaglandin E_2 increases survival in mice following irradiation. *Radiat. Res.,* 109, 440—448.

40. Weintraub, H., 1985: Assembly and propagation of repressed chromosomal states. *Cell,* 42, 705—711.

Received May 27, 2003

FOLIA VETERINARIA, 47, 4: 183—185, 2003

THE EFFECT OF WHOLE-BODY IRRADIATION ON THE CATECHOLAMINE LEVELS IN THE HYPOTHALAMUS OF SHEEP

Pástorová, B.

**University of Veterinary Medicine, Komenského 73, 041 81 Košice
The Slovak Republic**

pastorova@uvm.sk

ABSTRACT

The changes in the levels of catecholamines in the hypothalamus of sheep (n = 21) after whole-body irradiation with ^{60}Co at a total dose of 6.7 Gy were studied for seven days. A similar decrease in noradrenaline in the hypothalamus of sheep in comparison with the control was observed, most significantly (P < 0.001) in the rostral (by 74.2 %) and medial (P < 0.01) parts (by 40 %). A decline was found in dopamine, the levels of which decreased by 72 % in the rostral region, by 94 % in the medial (P < 0.001) one and by 60 % in the caudal hypothalamus. Our results have allowed us to presume that the whole-body irradiation of sheep decreased the content of catecholamines in the hypothalamus, most likely due to the failure of synthesis and degradation of catecholamines and to damage of the whole organism.

Key words: catecholamines; gamma irradiation; hypothalamus

INTRODUCTION

Knowledge about the effect of ionizing radiation on the hypothalamus is very important not only experimentally but especially in clinical practice. The therapeutic irradiation of cancer patients to the head certainly influences their neuroendocrine function and their central nervous system.

Ionizing radiation significantly changes the levels and metabolism of catecholamines as well as the amount of α and β-adrenergic receptors in different irradiated tissue (3, 4, 7, 8). The data of the effect of ionizing radiation on the metabolism of catecholamine in the brain available in the literature are various and depends on the kind of radiation, exposure dose and species sensitivity (9, 10).

The influence of ionising radiation on the hypothalamus of sheep has not been sufficiently described in the literature, which contains only some information obtained on laboratory animals (9, 10). Our study focused on the influence of ionising radiation on the catecholaminergic system of the sheep hypothalamus.

MATERIAL AND METHODS

Observations were carried out on twenty-one Slovak Merino sheep of a mean body weight of 44 ± 2,2 kg, two to three years old, in the physiological anoestrus (May). The first group (n = 6) served as a control. Sheep assigned to the second group (n = 6) were whole-body irradiated with ^{60}Co for seven days receiving a total dose of 6.7 Gy. The power input per hour of irradiation source was 0.039 Gy. After sheep decapitation the brain was removed from cranial cavity and the tissue was immediately, after its removal, immersed in liquid nitrogen to prevent degrading changes, it was kept in a frozen state until further processing. The *hypothalamus* was homogenized in Potter-Elvehjens glass microhomogenizator in 0.4 mol.l^{-1} $HClO_4$ with the addition of reduced glutation (0.05 mol.l^{-1}). The tissue homogenates were centrifuged at 20 000 *g*.min^{-1} at 0° C for thirty minutes. Chromatography on aluminium oxide according to Anton and Sayre (1) separated catecholamines from tissue supernatants. To eliminate the fluorescence of other components internal and external standards were prepared and blank tests of tissues and reagents were conducted. The results were reported as pg catecholamines.g^{-1} weight wet tissue in arithmetical means and processed statistically by a n-paired *t*-test.

RESULTS

Our results are presented in Tables 1–2. The application of a 6.7 Gy lethal dose of gamma rays significantly reduced the concentration of hypothalamic catecholamines (Tab. 1). Hypothalamic norepinephrine was present in the intact control sheep in a lower concentration than dopamine (Tab. 2). The concentration of dopamine (Tab. 1) declined throughout the hypothalamus after gamma-irradiation, being most pronounced ($P<0.001$) in the medial (by 94 %) and rostral (by 72.2 %) regions. A significant decrease ($P<0.01$) in dopamine level (by 60 %) was also observed in the caudal hypothalamus of the irradiated sheep. After seven-day irradiation of sheep with 6.7 Gy a decrease in the level of noradrenaline (Tab. 2) was observed in all the investigated hypothalamic parts, most significantly ($P<0.001$) in the rostral (by 74.2 %) and medial (by 40 %) regions ($P<0.01$).

Table 1. Dopamine (DA) levels in the *hypothalamus* of control and irradiated sheep

Hypothalamus	Controls $\bar{x}$ ± S.E.M. pg.g^{-1} DA	Irradiated $\bar{x}$ ± S.E.M. pg.g^{-1} DA
rostralis	240 ± 3.01	90 ± 1.80**
medialis	300 ± 5.00	20 ± 1.20**
caudalis	*280 ± 1.80*	*70 ± 3.00**

** — $p<0.001$ * — $p<0.05$

Table 2. Nradrenaline (NA) levels in the *hypothalamus* of control and irradiated sheep

Hypothalamus	Controls $\bar{x}$ ± S.E.M. pg.g^{-1} DA	Irradiated $\bar{x}$ ± S.E.M. pg.g^{-1} DA
rostralis	110 ± 3.5	40 ± 2.25 **
medialis	120 ± 4.1	85 ± 7.10 *
caudalis	160 ± 4.3	170 ± 3.00

** — $p<0.001$ * — $p<0.01$

DISCUSSION

Hypothalamic catecholamines participate in the regulation of several neuroendocrine processes. They play an important role in the regulation of sexual functions and in stress responses. Because the literary data on the influence of ionising radiation on catecholamine levels in the brain of sheep are scarce, we can compare our results only with those obtained with laboratory animals.

It is known that catecholamines of the brain, hypothalamus, heart and other tissues of laboratory animals are significantly decreased after exposure to ionizing whole body irradiation (2, 3, 4, 9, 10). The turnover and actual levels of catecholamines in the nerve tissue depend on other factors such as storage and uptake, absorption from trans-neural flux and interaction with auto-receptors (10). The injury of some of the given factors with radiation leads to changes in the concentrations and functions of catecholamines in the nerve tissue (22) and these changes occur in the early and prolonged period of post-irradiation.

The whole-body X-ray irradiation of rats disturbed their metabolism and catecholamine function in the entire brain, hypothalamus and adrenal glands (2). Stepanovic *et al.* (8) have observed a marked decrease in catecholamines in the hypothalamus, *corpus striatum,* heart and brain of rabbits and rats exposed to whole-body X-ray irradiation. Our results were similar, indicating a significant decrease in catecholamines in the hypothalamus of sheep irradiated with a total dose 6.7 Gy for seven days.

In accordance with the results of other authors (2, 9, 10) we have assumed that the reduction in dopamine and norepinephrine levels in the hypothalamus can be related to disturbances in metabolic processes in the organism as a whole, damage to vessel endothelium and the subsequent decrease or restriction of catecholamine synthesis. This suggests that besides metabolic damage to animals during their gamma-irradiation the mechanisms of uptake and storage of catecholamines may be affected, too (2, 8).

In our previous work (4) after whole-body, five days continuous irradiation of sheep with a daily dose of 0.5 Gy up to an accumulated dose of 2.5 Gy gamma rays a significant decrease in hypothalamic norepinephrine and dopamine was found 120 hours after exposure. Based upon our previous results, its follows that the lethal protracted gamma irradiation (14.35 Gy) in rat pineal gland decreased norepinephrine and dopamine pineal levels 30 and 120 minutes after exposure. Stepanovic *et al.* (10) have found that irradiated rats with doses 650 and 850 R are able to metabolise a precursor of catecholamine synthesis l-DOPA and store the new-synthesized catecholamines in their nerve tissue, when the activity of peripheral DOPA-decarboxylase is inhibited by benserazide.

In addition to the facts mentioned, the decrease in catecholamine levels, observed in the hypothalamus, may be associated with an increase in the activity of the enzymes responsible for catecholamine degradation in the brain of rabbits and ewes observed after exposure (2, 6).

REFERENCES

1. Anton, A. H., Sayre, D. F., 1972: Fluorometric assay of catecholamines, serotonin and their metabolites. In **Rall, J. E.,**

Kopin, I. J. (ed.): *Method in Investigative Endocrinology.* North Holland Publishing Comp., Amsterdam, London, 398—436.

2. Dahlström, A., Haggendal, J., Rosenberg B., 1983: Catecholamines in hypothalamus of X-irradiated rats. *Acta Radiol. Ther.,* 12, 191—200.

3. Pástorová, B., Ahlersová, E., Ahlers, I., Várady, J., 1997: The effect of a single whole-body irradiation with a lethal dose on catecholamine levels in the pineal glands of rats. *J. Physiol. Pharmacol.,* 48, 75—82.

4. Pástorová, B., Arendarčik, J., 1998: Effect of protracted exposure to gamma radiation and hormonal stimulation with Folistiman (FSH) on the content of catecholamines in the hypothalamus and hypophysis of ewes. *Vet. Med.* (Praha), 33, 209—217.

5. Pástorová, B., Arendarčik, J., 1989: Effect of protracted exposure to gamma radiation and hormonal stimulation with serum gonadotrophine on the content of catecholamines in the hypothalamus, epiphysis and adrenal glands of ewes. *Vet. Med.* (Praha), 34, 171—180.

6. Pástorová, B., Arendarčik, J., 1988: The effect of protracted exposure to gamma radiation (6.7 Gy) on the activity of monoaminooxidase in the hypothalamus of ewes in the anoestral period. *Vet. Med.* (Praha), 33, 735—740.

7. Reiter, R. J., Richardson, B. A., 1992: Magnetic field effect on pineal indolamine metabolism and possible biological consequences. *FASEB J.,* 6, 2283—2287.

8. Stepanovič, S. R., Nikolič, J. V., Varagič, V. M., 1981: The effect of l-DOPA and benserazide on the amount of catecholamines in heart atria, hypothalamus and corpus striatus **o**f X-irradiated rats. In **Usdin, E., Kopin, I. J.** (ed.): *Catecholamines and Stress.* Elsevier North Holland Elsevier North Holland Recent Advances, New York, Amsterdam, Oxford.

9. Timmermans, S., Gerber, G. B., 1984: The effect of X-irradiation on cardiaciac beta-adrenergic receptors in the rabbit. *Radiat. Res.,* 100, 510—518.

10. Walden, T. L., Farzaneh, N., 1989: Biogenic amines. In **Walker, R., Cervaney, J. J.** (ed.): *Biochemistry of Ionizing Radiation.* Raven Press Ltd., 1185 Avenue of the American, New York, Office of the Surgeon General, 55—88.

Received September 22, 2003

FOLIA VETERINARIA, 47, 4: 186—187, 2003

PATHOGENESIS OF POST-IRRADIATION APLASTIC ANAEMIA IN CHICKENS AND ITS DIAGNOSIS

Sesztáková, E., Beňová, K., Škardová, I.
Falis, M., Toropila, M., Fialkovičová, M.

University of Veterinary Medicine, Komenského 73, 041 81 Košice
The Slovak Republic

sesztakova@uvm.sk

ABSTRACT

We have evaluated the haematological parameters of the red blood cells in 28-day old broiler chickens irradiated with gamma rays using a single whole-body dose of 5.0 Gy at the dose rate 0.27 Gy.min^{-1}. Blood samples were taken from the *vena cutanea ulnaris* at 6, 24, 48, and 72 hour intervals post-irradiation. We have evaluated the total red cells, haemoglobin, haematocrit, erythrocyte indices and the results have been compared with those obtained from the control group. Statistically significant values ($p < 0.05$) were obtained only for the haematocrit 48 and 72 hours post-irradiation. At the dose of radiation we used the haematological parameters did not show a marked picture of aplastic anaemia which is accompanied by post-irradiation disease.

Key words: anaemia; chickens; gamma radiation; indices of erythrocytes; red blood cells

INTRODUCTION

It is generally known that red blood cells are more radio-resistant compared with white blood cells. Because of the large deposits of erythrocytes in the spleen and liver, the total erythrocyte count declines gradually after irradiation treatment.

After the use of high doses of radiation or long term radiation the total erythrocyte count, haemoglobin content and haematocrit declines. Besides these changes, we have also determined the erythrocyte indices for the evaluation of anaemia (mean corpuscular volume – MCV, mean corpuscular haemoglobin – MCH, mean corpuscular haemoglobin concentration – MCHC).

Post-irradiation aplastic anaemia develops as a consequence of pluripotent cell disorder (a decrease in the pluripotent cell count, a defect in pluripotent cells) and micro-enviromental disorder in pluripotent cells (destruction in the bone marrow structure and its micro-circulation) (1).

In the following experiment we tried to observe the change in the dynamics of the developmental stages of red blood cells in broiler chickens irradiated with a single whole-body dose of 5.0 Gy.

MATERIAL AND METHODS

In the experiment thirty 28-day old broiler chickens ($n = 6$ in each group) were used. Before the experiment, the chickens were kept for one week in a previously disinfected room to acclimatize them to experimental conditions (3). They were supplied standard feed and water *ad libitum*. Their rations consisted of BR 1 commercial granulated feed. The animals were exposed to a single dose of whole-body gamma radiation of 5.0 Gy (Chisostat, ^{60}Co-sourse, Chirana) directly in the adjusted plexit cages. The control chickens were exposed to sham irradiation, i.e. they were handled in the same way as the experimental ones except for the irradiation. After which they were irradiated with gamma rays using a single whole-body dose of 5.0 Gy at the dose of 0.27 Gy.min^{-1}. Blood samples were taken from the *vena ulnaris cutanea* before feeding at 6, 24, 48, and 72 hours intervals post-irradiation. Haematological examination and indices of erythrocytes were carried out using common methods (2).

The experiment was conducted in summer.

The results obtained were evaluated in comparison with the control using Student's *t*-test.

RESULTS AND DISCUSSION

The values of haematological parameters and indices of erythrocytes are shown in Table 1.

Table 1. Haematological parameters and indices of erythrocytes in broiler chickens after gamma irradiation

	RBC ($T.l^{-1}$)	Hb ($g.l^{-1}$)	PVC ($l.l^{-1}$)	MCHC ($mmol.l^{-1}$)	MCH (fmol)	MCV (fl)
			6 h after irradiation			
$\bar{x}$	1.79	90.67	0.30	18.16	32.32	176.18
SD ±	0.25	6.76	0.02	0.60	3.62	1.47
			24 h after irradiation			
$\bar{x}$	1.89	97.87	0.33	18.33	32.14	167.59
SD ±	0.14	8.90	0.01	2.38	5.00	16.33
			48 h after irradiation			
$\bar{x}$	1.71	92.27	0.32*	17.34	33.48	192.26
SD ±	0.12	7.62	0.01	0.06	1.94	10.95
			72 h after irradiation			
$\bar{x}$	1.64	90.93	0.36*	18.21	34.35	194.59
SD ±	0.12	11.20	0.00	0.00	2.44	0.00
			Control			
$\bar{x}$	2.01	97.60	0.34	18.36	30.85	170.66
SD ±	0.44	8.28	0.01	1.06	4.68	28.44

** — $P < 0.05$*

We observed a decrease in the total RBC count and in the haemoglobin concentration at all intervals compared with the control group. But this decrease in count was statistically insignificant. There was a significant difference ($P < 0.05$) between haematocrit value after 48 hours radiation treatment when the haematocrit value decreased and 72 hours post-irradiation when the level was increased.

Similar changes in the red blood cells' picture as presented in our experiment after using single whole-body dose gamma irradiation of 5.0 Gy, has also been described by other authors (4, 5, 9) with different doses of gamma irradiation. A lower radio-sensitivity of red blood cells compared with white blood cells has also been described by these authors.

For the qualitative evaluation of abnormalities in erythropoesis, the results of the indices of erythrocytes, MCHC and MCH are presented, which show an increase in all experimental groups in comparison with the control group. In MCV values we recorded a decline after 6 and 24 hours of post-irradiation intervals and an increase after 48 and 72 hours of post-irradiation intervals in comparison with the control group. These changes were statistically insignificant.

There are few reports on post-irradiation indices of erythrocytes in poultry.

Škardová (10) has described changes in erythrocytes indices using cumulative doses from 2.7 to 28.6 Gy which can be specific for the character of anaemia. Erythrocyte indices were also studied by Malhotra *et al. (6, 7) using single whole-body dose gamma radiation of 2.5 Gy. These authors have reported higher values of erythrocytes indices compared with the values obtained by us. They have stated that the elevated MCV values may be due to the changed permeability in the cell membrane of the irradiated erythrocytes which evidently absorb large amounts of water and become swollen. This change appears to be basic to the alteration observed in MCH and MCHC values.*

In the evaluation of post-irradiation changes in the red blood cells' picture we must consider these changes as a result of abnormality in erythropoesis in the bone marrow (8).

Changes in RBC after the dose of gamma radiation used in our study were not so expressive, but we consider that after 7—14 days post-irradiation intervals it will be possible to record characteristic changes of aplastic anaemia which is accompanied by radiation sickness.

REFERENCES

1. Friedman, B., 1994: *Haematology in Praxis* (In Czech). Galen, 394 pp.

2. Gaál, T., 1999: *Veterinary Clinical Laboratory Diagnostics* (In Hungarian). SIK, Budapest, 490 pp.

3. Kubíček, K., Novák, P., Kočišová, A., Rodl, P., 2000: *Disinfection, Disinfectization and Rat Control in Schemes, Tables and Figures* (In Czech). Veterinary and Pharmaceutical University, Brno, 100 pp.

4. Lee, S. W., Ducoff, H. S., 1994: The effects of ionizing radiation on avian erythrocytes. *Radiation Reserch,* 137, 104—110.

5. Malhotra, N., Rana, K., 1988: Effect of gamma radiation on haematology of chick *(Gallus gallus domesticus). Radiobiol. Radiother.,* 29, H 1, 119—132.

6. Malhotra, N., Rana, K., Malhotra, R. K., 1989: Haematocytometrical changes in chicken blood to acute ^{60}Co gamma radiation. *Indian J. Exp. Biol.,* 27, 1106—1108.

7. Malhotra, N., Rani, N., Rana, K., Malhotra, R. K., 1990: Radiation induced blood pathology in chick-erythrocytes and related parameters. *Exp. Pathol.,* 38, 241—248.

8. Sesztáková, E., Beňová, K., Škardová, I., Toropila, M., Leistein, R., 1999: Post-irradiation changes in myelogram of chickens. *Folia veter.,* 43, 196—199.

9. Škardová, I., Gavalec, M., 1991: Radioprotection of the chickens, analysis of clinical and haematological parameters (In Slovak). *Živočíšna výroba,* 36, 895—901.

10. Škardová I., 1992: Post-irradiation changens of the chickens (In Slovak). *Conference of Poultry,* Stará Lesná, SR, 168—171.

Received June 16, 2003

FOLIA VETERINARIA, 47, 4: 188—192, 2003

THE HAEMATOLOGICAL PROFILE OF BROILERS UNDER ACUTE AND CHRONIC HEAT STRESS AT 30 ± 1 °C LEVEL

Bedáňová, I., Voslářová, E., Večerek, V., Straková, E., Suchý, P.

University of Veterinary and Pharmaceutical Sciences Brno
Palackého 1-3, 612 42 Brno
The Czech Republic

bedanovai@vfu.cz

ABSTRACT

Changes in selected haematological criteria in broilers under acute and chronic heat stress have been monitored with the aim of gaining a better understanding of the influence of high temperatures on fattened broilers. The indices monitored included total erythrocyte and leukocyte counts, haematocrit value and haemoglobin levels in broiler blood. The differential leukocyte counts have also been calculated. These indices have been evaluated separately for male and female broilers. The results for male broilers show a significant decrease in haematocrit values (P = 0.011) and haemoglobin levels (P = 0.000) following long-term exposure to high ambient temperatures (30 ± 1 °C throughout the fattening period). On the other hand for female broilers under chronic heat stress, no significant changes in haematological indices were observed. In the differential leukocyte count, chronic heat stress caused a highly significant decrease in lymphocyte count (P = 0.001 and 0.006 in females and males, respectively) and subsequently also in the higher H/L ratio, which was also highly significant (P = 0.009 and 0.000 in females and males, respectively). Acute heat stress at the age of forty-two days (30 ± 1 °C for 24 h) caused a significant decrease in haematocrit values in both female and male broilers (P = 0.012 and 0.037, respectively). Females, moreover, demonstrated a significant decrease in haemoglobin levels (P = 0.023) and total erythrocyte count (P = 0.048). The differential leukocyte count calculated after acute heat stress shows a decrease in heterophil granulocytes in male broilers (P = 0.026) and also a decreased H/L ratio that is highly significant (P = 0.009). In both female and male broilers, acute heat stress causes a significant increase in basophil granulocytes (P = 0.037 and P = 0.048, respectively).

Key words: haematocrit; haemoglobin; leukocyte; heterophil/lymphocyte ratio

INTRODUCTION

Ambient temperature is one of the most important factors in the external environmental for the fattening of broilers because it may significantly affect their metabolism and, subsequently, the efficiency of the fattening process. In their study of effects of high ambient temperatures on broilers, Večerek *et al.* (6) have reported depressed growth and significant changes in the internal environment for chickens due to gradually increasing ambient temperatures that started on day 16 of the fattening period, specifically decreased haemoglobin levels and increased total blood leukocyte counts. Yahav and Hurwitz (7) have described decreased haematocrit values in fattened broilers in connection with a 24-hour exposure to 36 ± 1 °C early in life. Decreased haematocrit values in broilers following exposure to heat during fattening have also been reported by Zhou *et al.* (8). Altan *et al.* (1) believe that acute heat stress causes no changes in haematocrit values.

The differential leukocyte count and the monitoring of its changes in relation to effects of heat stress is an important part of the haematological profile of broilers. Maxwell and Robertson (3) have pointed out that the number of heterophils in chicken blood generally exceeds that of lymphocytes until one week after hatching and that later the H/L ratio becomes lower. Because moderate exposures to most stressors will cause an increase in the number of heterophils, the H/L ratio may be used as a stress level indicator. Similar conclusions have been reached by McFarlane and Curtis (4) who have found that a 7-day increase in ambient temperatures (30.4 and 34.8 °C) caused an increase in the H/L ratio in female broiler chickens and that the leukocyte ratio changes are less variable and more stable than plasma corticosterone levels. They claim that in some cases the H/L ratio is a more reliable stress load indicator than plasma corticosterone concentrations. An increase in the H/L ratio caused by chronic heat stress in

broilers has also been reported by McKee and Harrison (5) and Zulkifli *et al.* (9).

The effects of acute heat stress on the ratios between individual types of leukocytes in broiler chickens have been monitored by Altan *et al.* (1), who have found that an increase in ambient temperature to 39 ± 1 °C for two hours causes an increase in the heterophil/basophil ratio, an increase in the H/L ratio from 0.25 to 0.43 and a decrease in the monocyte / lymphocyte ratio. The number of eosinophils are not affected.

Maxwell (2) has found heteropenia and basophilia develop in fowl under extreme stress and the H/L ratio can therefore be an accurate stress indicator only in the case of weak to mild stress. He believes that birds especially may have a two-stage leukocyte response to stress loads. Maxwell and Robertson (3) have also claimed that the H/L ratio in chickens can be used as a reliable indicator in physiological stress because high stress loads may cause heteropenia.

MATERIAL AND METHODS

A group of COBB 500 broilers was used to study the effects of chronic heat stress on the haematological profile of meat hybrid poultry breeds. From the first day after hatching, both the test group (forty male and forty female chickens) and the control group (forty male and forty female chickens) were housed separately according to sex on deep litter in an experimental barn with controlled light, heat, hygienic and feeding patterns. Both groups received feed and water *ad libitum*. The test group was kept at a constant ambient temperature of 30 ± 1 °C. The temperature was constantly monitored and recorded by digital thermometers. Relative humidity throughout the test period was between 20 % and 40 %. For the control group, ambient temperatures were gradually decreased from 30 ± 1 °C on Day 1 of fattening to 20 ± 1 °C on the last day of fattening (Day 42). Depending on ambient temperatures, relative humidity levels ranged between 20 % and 60 %. When the broilers were forty-two days old, ten female chickens and ten male chickens were picked at random from both the test and the control groups and blood samples were drawn from the *vena basilica*. For haematological examinations, the samples were stabilized by heparin.

The effects of acute heat stress on the haematological profile of meat hybrid poultry breeds were studied using ROSS 308 broilers. From the first day after hatching, a group of forty broilers (twenty female and twenty male chickens) were housed separately according to sex on deep litter in an experimental barn light, heat, hygienic and feeding patterns. The broilers were given feed and water *ad libitum*. Ambient temperatures were continuously monitored and recorded. The ambient temperature was gradually decreased from 30 ± 1 °C on Day 1 of fattening down to 20 ± 1 °C on the last day of fattening (Day 42). On Day 42, the ambient temperature was increased to 30 ± 1 °C and maintained at that level for twenty-four hours, at relative humidity of 26 ± 1 %. Before the ambient temperature increase, ten female and ten male chickens were picked at random and blood samples were taken from their *vena basilica.* The same broilers had blood samples taken the after 24-hour period of the raised ambient temperature. For haematological examinations, the samples were stabilized by heparin.

In this study of chronic and acute heat stress effects on the haematological profile, haematological examinations consisted in determining total erythrocyte and leukocyte counts, haematocrit values, haemoglobin levels and differential leukocyte counts, whereby the proportions of neutrophil, basophil and eosinophil granulocytes, lymphocytes and monocytes of the total leukocytes were computed. The total erythrocyte and leukocyte counts were determined by means of the flask method of dilution and counting corpuscles using the Bürker chamber, haemoglobin levels were determined photometrically using the SPECOL-11 photometer and Drabkin's solution at a 540 nm wavelength, and haematocrit values were determined by means of the micro-haematocrit technique according to Janetzki. The proportions of individual leukocyte types were computed from blood smears panoptically stained according to Pappenheim with a use of microscope with an immersion lens. Results were statistically processed using the Unistat 5.1 software. Differences between the means of individual haematological indices were calculated for female and male broiler chickens separately and subjected to the *t*-test.

RESULTS

The results of haematological examinations of female and male broiler chicken under chronic heat stress are given in Table 1.

It follows from the results that a long-term increase in the ambient temperature during fattening had no statistically significant effect in female broiler chickens in the indices monitored. In male broiler chickens, however, the haematocrit value and haemoglobin levels showed a significant decrease following a long-term increase in ambient temperature (P = 0.011 and P = 0.000, respectively). The mean values of the total erythrocyte

Table 1. Changes in haematological indices in broiler chickens under chronic heat stress

		ERY [T.l^{-1}]	LEU [G.l^{-1}]	HK [l.l^{-1}]	Haem [g.l^{-1}]
		Females			
Control	Mean	2.240	14.800	0.312	88.823
	(SD)	(0.327)	(2.163)	(0.036)	(8.880)
Stressed	Mean	2.106	13.500	0.290	84.284
	(SD)	(0.267)	(4.163)	(0.014)	(8.603)
		–	–	–	–
t-test	P	0.329	0.392	0.094	0.261
		Males			
Control	Mean	2.420	16.800	0.308	91.292
	(SD)	(0.586)	(5.453)	(0.033)	(10.539)
Stressed	Mean	2.033	13.300	0.274	73.135
	(SD)	(0.591)	(3.882)	(0.013)	(4.116)
		–	–	*	**
t-test	P	0.159	0.116	0.011	0.000

ERY — total erythrocyte count; LEU — total leukocyte count; HK — haematocrit value; Haem — haemoglobin level
* — P < 0.05; ** — P < 0.01

and leukocyte counts in male broiler chickens from the test group were lower compared with the controls, the difference, however, was not significant.

Changes in the differential leukocyte count in the blood of female and male broiler chickens under chronic heat stress are given in Table 2.

Table 2. Changes in differential leukocyte count in broiler chickens under chronic heat stress

		Het [G.l^{-1}]	Ly [G.l^{-1}]	Eos [G.l^{-1}]	Bas [G.l^{-1}]	Mo [G.l^{-1}]	H/L
				Females			
Control	Mean	5.83	8.57	0.13	0.20	0.07	0.69
	(SD)	(1.487)	(1.31)	(0.04)	(0.06)	(0.01)	(0.20)
Stressed	Mean	7.68	5.50	0.10	0.14	0.07	1.57
	(SD)	(2.58)	(2.17)	(0.04)	(0.06)	(0.02)	(0.83)
		–	**	–	–	–	**
t-test	P	0.064	0.001	0.183	0.065	0.392	0.009
				Males			
Control	Mean	7.02	9.25	0.22	0.20	0.10	0.78
	(SD)	(2.80)	(3.09)	(0.15)	(0.10)	(0.06)	(0.24)
Stressed	Mean	7.39	5.46	0.13	0.14	0.07	1.43
	(SD)	(1.80)	(2.26)	(0.04)	(0.06)	(0.02)	(0.26)
		–	**	–	–	–	**
t-test	P	0.725	0.006	0.090	0.106	0.096	0.000

Het — heterophils; Ly — lymphocytes; Eos — eosinophils; Bas — basophils; Mo — monocytes; H/L — heterophil/lymphocyte ratio
* — P < 0.05; ** — P < 0.01

It follows from the results that a long-term increase in ambient temperatures during the fattening period caused a highly significant drop in the number of lymphocytes in both female and male broiler chickens (P = 0.001 and 0.006, respectively), and a highly significant increase in the H/L ratio in both female and male broiler chickens (P = 0.009 and 0.000, respectively). The mean neutrophil granulocyte (heterophil) counts were raised more markedly in female chickens than in male compared with the control group, but neither of the differences was significant. Other parameters, i.e. the eosinophil and basophil granulocyte counts and the monocyte count, showed no significant differences between the test and the control groups, or between female or male broiler chickens.

The results of the haematological examinations of female and male broiler chickens under acute heat stress are given in Table 3.

It follows from the table that an increase in ambient temperatures to 30 ± 1 °C for 24 hours caused a significant decrease in haematocrit vales in both female and male 42-day old broiler chickens (P = 0.012 and 0.037, respectively). Female broiler chickens, moreover, showed a significant decrease in the mean haemoglobin level (P = 0.023) and in the total erythrocyte count (P = 0.048). In male broiler chickens, changes in the two indices compared with the control group were not significant.

Changes in the differential leukocyte count in the blood of female and male broiler chickens under acute heat stress are given in Table 4.

Table 3. Changes in haematological indices in broiler chickens under acute heat stress

		ERY [T.l^{-1}]	LEU [G.l^{-1}]	HK [l.l^{-1}]	Haem [g.l^{-1}]
			Females		
Before	Mean	1.908	18.750	0.272	80.628
stress	(SD)	(0.242)	(2.927)	(0.019)	(8.916)
Stressed	Mean	1.716	21.100	0.250	74.121
	(SD)	(0.028)	(0.354)	(0.028)	(4.271)
		*	–	*	*
t-test	P	0.048	0.292	0.012	0.023
			Males		
Before	Mean	1.882	16.850	0.282	86.488
stress	(SD)	(0.190)	(3.283)	(0.015)	(12.329)
Stressed	Mean	1.936	18.050	0.266	82.610
	(SD)	(0.277)	(5.320)	(0.021)	(8.887)
		–	–	*	–
t-test	P	0.423	0.362	0.037	0.455

ERY — total erythrocyte count; LEU — total leukocyte count; HK — haematocrit value; Haem — haemoglobin level
* — P < 0.05; ** — P < 0.01

It follows from the above that an increase in ambient temperature to 30 ± 1 °C for 24 hours causes in male broiler chickens a significant decrease (P = 0.026) in the number of neutrophil granulocytes (heterophils) and, subsequently, a decrease in the H/L ratio, which was highly significant (P = 0.009). Under acute heat stress, basophilia was found in chicken of both sexes. The increase in the number of basophilic granulocytes in female and male broiler chickens was significant (P = 0.037 and 0.048, respectively), the average increase in male broiler chickens was more than twofold. A similar downward trend in the heterophil granulocyte counts and the H/L ratio was found in female broiler chickens, but the dif-

Table 4. Changes in differential leukocyte count in broiler chickens under acute heat stress

		Het [G/l^{-1}]	Ly [G/l^{-1}]	Eos [G/l^{-1}]	Bas [G/l^{-1}]	Mo [G/l^{-1}]	H/L
				Females			
Before	Mean	5.87	12.42	0.17	0.20	0.09	0.48
stress	(SD)	(0.97)	(2.25)	(0.07)	(0.07)	(0.01)	(0.08)
Stressed	Mean	5.68	14.87	0.13	0.28	0.11	0.40
	(SD)	(3.09)	(4.35)	(0.07)	(0.05)	(0.03)	(0.21)
		–	–	–	*	–	–
t-test	P	0.837	0.145	0.153	0.037	0.302	0.210
				Males			
Before	Mean	5.56	10.84	0.18	0.19	0.08	0.54
stress	(SD)	(1.45)	(2.52)	(0.07)	(0.08)	(0.02)	(0.19)
Stressed	Mean	3.60	13.68	0.15	0.52	0.10	0.29
	(SD)	(1.73)	(4.74)	(0.08)	(0.41)	(0.06)	(0.13)
		*	–	–	*	–	**
t-test	P	0.026	0.191	0.474	0.048	0.391	0.009

Het — heterophils; Ly — lymphocytes; Eos — eosinophils; Bas — basophils; Mo — monocytes; H/L — heterophil/lymphocyte ratio
* — P < 0.05; ** — P < 0.01

ferences between mean values before and after the heat stress exposure were not significant.

DISCUSSION

The results of the experiment show that an increase in ambient temperature may have a significant impact on the haematological profile in broilers and these effects of long-term and short-term heat stress are different, both from the point of view of individual haematological indices and differences between female and male broiler chickens.

In our study of long-term high temperature effects on haematological indices in broilers, male chickens seemed to be more affected than female chickens. While no significant differences between female broiler chickens and their control counterparts fattened at normal temperatures were found, male broiler chickens showed a drop in haematocrit values and haemoglobin levels. A decrease in haematocrit values due to heat exposure in the period of fattening have also been found by Zhou *et al.* (8), who, however, did not distinguish between sexes. A decrease in total haemoglobin level is in agreement with the conclusions of Večerek *et al.* (6); in the present experiment, however, the decrease has been demonstrated in male broilers only. Changes in the differential leukocyte count under chronic heat stress has shown a marked increase in the H/L ratio in both male and female broiler chickens, which corresponds with data reported by McKee and Harrison (5), Zulkifli *et al.* (9) and McFarlane and Curtis (4).

In our study, the increase in the H/L ratio was mainly due to the decrease in lymphocyte counts and also to a slight increase in heterophil counts. Maxwell and Robertson (3), on the other hand, have hypothesized that an increase in the H/L ratio in chickens under heat stress is attributable mainly to the growing numbers of heterophils under stress.

The results of our study into the effects of acute heat stress on haematological indices in broilers indicate that even a short-term increase in ambient temperature will cause changes in the haematological profiles of both female and male broiler chickens. These changes differ in character from those caused by chronic heat stress. This is particularly true of differential leukocyte counts.

We have found that acute, as opposed to chronic, heat stress causes a decrease in the number of heterophil granulocytes and, subsequently, in the H/L ratio, which, however, was statistically significant in male chickens only. There was a marked increase in basophil counts which was significant in both male and female broiler chickens. These results correspond to conclusions reached by Maxwell and Robertson (3), who have reported the development of heteropenia and basophilia as a result of acute extreme stress in poultry.

Altan *et al.* (1), on the other hand, have observed increased H/L and heterophils-to-basophils ratios in broilers under acute heat stress. The same authors have found no change in the haematocrit value as a result of acute heat stress, which is contrary to our findings of a statistically significant decrease in haematocrit values in both male and female broiler chickens. A similar decrease in haematocrit values has been observed by Yahav and Hurwitz (7). The results of other haematological tests in our study show that acute heat stress causes a decrease in erythrocyte counts and total haemoglobin levels, which is particularly noticeable in female chickens.

CONCLUSION

Changes in differential leukocyte counts seem to be an important and specific indicator for heat stress intensity in broilers, exposed to the temperature 30 ± 1 °C for both a short and long time period. Under acute stress, the changes are characterized by the development of basophilia, which is more pronounced in male broilers who, contrary to female broiler chickens, also show a statistically highly significant decrease in H/L ratios. Chronic heat stress, on the other hand, is characterized by an increase in H/L ratios, which is highly significant in both sexes. This seems to corroborate the hypothesis of a two-stage response to heat stress in broilers proposed also by Maxwell (2), who believes that it may be a response specific to avian species.

Acknowledgements

This research was supported by the Ministry of Education, Youth and Sports of the Czech Republic (MSM Project No. 162700004).

REFERENCES

1. Altan, O., Altan, A., Cabuk, M., Bayraktar, H., 2000: Effects of heat stress on some blood parameters in broilers. *Turk. J. Vet. Anim. Sci.,* 24, 145—148.

2. Maxwell, M. H., 1993: Avian blood leukocyte responses to stress. *World Poultry Sci. J.,* 49, 34—43.

3. Maxwell, M. H., Robertson, G. W., 1998: The avian heterophil leukocyte: a review. *World Poultry Sci. J.,* 54, 155—178.

4. McFarlane, J. M., Curtis, S. E., 1989: Multiple concurent stressors in chicks. 3. Effects on plasma corticosterone and the heterophil:lymphocyte ratio. *Poultry Sci.,* 68, 522—527.

5. McKee, J. S., Harrison, P. C., 1995: Effect of supplemental ascorbic acid on the performance of broiler chickens exposed to multiple concurrent stressors. *Poultry Sci.,* 74, 1772—1785.

6. Večerek, V., Straková, E., Suchý, P., Voslářová, E., 2002: Influence of high environmental temperature on production and haematological and biochemical indexes in broiler chickens. *Czech J. Anim. Sci.,* 47, 176—182.

7. Yahav, S., Hurwitz, S., 1996: Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. *Poultry Sci.,* 75, 402—406.

8. Zhou, W. T., Fujita, M., Yamamoto, S., 1999: Thermoregulatory responses and blood viscosity in dehydrated heat-exposed broilers *(Gallus Domesticus). J. Therm. Biol.,* 24, 185—192.

9. Zulkifli, I., Dunnington, E. A., Gross, W. B., Siegel, P. B., 1994: Inhibition of adrenal steroidogenesis, food restriction and acclimation to high ambient temperatures in chickens. *Brit. Poultry Sci.,* 35, 417—426.

Received October 13, 2003

FOLIA VETERINARIA, 47, 4: 193—196, 2003

THE OCCURRENCE OF CANINE VOGT-KOYANAGI-HARADA AS A SYNDROME IN THE ROTTWEILER (A Case Report)

Trbolová, A.[1], Kozák, I.[2], Ševčíková, Z.[1]
Balicky, I.[3], Ledecký, V.[1], Capík, I.[1]

[1]University of Veterinary Medicine, Komenského 73, Košice
[2]P.J.Šafárik University, Faculty of Medicine, Trieda SNP 1, Košice
The Slovak Republic
[3] University of Veterinary Medicine, Głęboka 30, Lublin
Poland

trbolova@uvm.sk

SUMMARY

We have reported an unusual occurrence of uveodermatologic syndrome in a Rottweiler dog (3.5 years old male animal). To our knowledge, this is the first description of such a syndrome in this breed. Diagnosis was based on both clinical and histopathologic examinations. Typical ocular changes included, slight corneal oedema, bilateral uveitis with posterior synechiae, cataract, secondary glaucoma and choroidal depigmentation. Other accompanying dermatologic changes included depigmentation, lichenoid dermatitis of *dorsum nasi*, scrotum and footpads which firmly supported our diagnosis. This was also confirmed by the histopathology of dermal lesion. The therapeutic outcome in a reasonably long follow-up period was impossible to observe because the owner decided to have the dog put down two weeks after the diagnosis had been made.

Key words: dog; Rottweiler; Vogt-Koyanagi-Harada-like syndrome

INTRODUCTION

The syndrome characterized by *uveitis, alopecia, vitiligo, poliosis* and *dysacusis* was first described in human medicine by Vogt in 1906 (17) and later Koyanagi in 1914 (9). Harada 1926 (6) contributed with the clinical description of retinal detachment and pleiocytosis of cerebrospinal fluid.

In 1977, Asakura, Takahashi and Onishi (1) reported detailed clinical findings affecting two Akita dogs in Japan. Since that time the literature has described this disease as a Vogt-Koyanagi-Harada (VKH)-like syndrome or uveodermatologic (UD) syndrome in dogs. Such a recognition brought about more reports on this clinical entity. For instance, in 1982 Canadian Bussanich *et al.* (2) described a granulomatous panuveitis and depigmentation affecting two Samoyeds and Irish Setter. This disease was also observed by the French in a Siberian Husky by Fabries in 1984 (5); in the United States Romatowsky (15) first referred to uveodermatologic syndrome in Akita-Inu; Kern *et al.* (8) in a Golden Retriever, Campbell *et al.* (3) in Collie and in a Dachshund Herrer and Duchene (7).

Cardinal ophthalmologic findings in dogs with this disease include bilateral anterior/posterior uveitis and/or *panuveitis* in addition to retinal detachment. As a consequence, there is the formation of a cataract (10). The most frequent dermatologic changes (depigmentation) appear in the nasal, mouth, eyelid, periorbital areas, in bucal mucosa, footpads and testes.

In this article we provide a clinical and histopathologic description of a unique occurrence of UD syndrome in a Rottweiler dog. Differential diagnosis, treatment options and prognosis are discussed.

A CASE DESCRIPTION

History

A 3.5 year old Rottweiler male dog, was referred to our clinic due to vision abnormalities and facial dermatosis which had existed for three months. Previous topical therapy with antibiotics and corticosteroids had not produced any clinical

improvement. During the previous month the dog had developed conjunctival hyperaemia with severe signs of blefarospasm and photophobia. Erythema, depigmentation and ulceration of the nasal planum, lips, scrotum and foot pads had also occurred. The labial mucosa and soft palate were ulcerated. The dog had been unable to find its way in any unknown environment.

Clinical signs

Ophthalmic examination

On ophthalmic examination, all abnormalities were bilateral. They included negative menace response, palpebral and corneal reflexes were exaggerated, plus there was no pupillary reflex due to *synechiae*. Slit lamp examination was performed to evaluate changes in the anterior part of the globe. Examination revealed slight corneal oedema, accompanied by hyperemia of the ciliary vessels. The iris was also congested with a granular appearance. Accompanied with signs of *dyscoria* and posterior *synechiae* (Fig. 1). The lenses were cataractous. On fundoscopy, which was impaired due to cataract, we found focal chorioidal

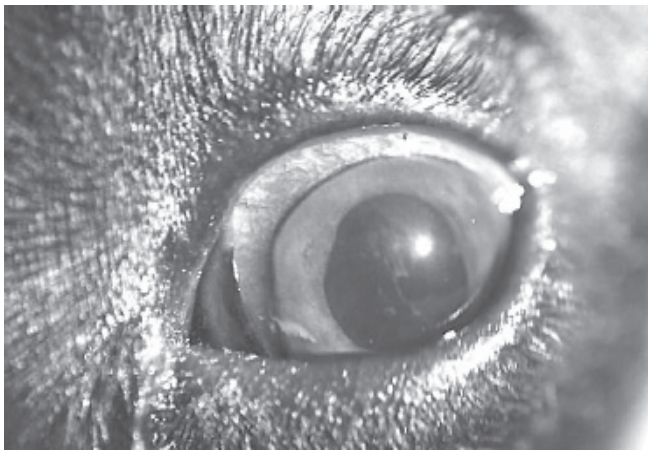


Fig. 1. Slight corneal oedema, hyperemia of the ciliari vessels, congestion of the iris, dyscoria and posterior synechiae

were obtained from the outer margin of the depigmented and eroded areas of the nose and scrotum. They were fixed in 10 % formalin and were routinely processed for haematoxylin and eosin staining which was later examined by direct immunofluorescence.

Histopathologic examination showed an irregular epidermal hyperplasia with orthokeratotic and parakeratotic hyperkeratosis with lichenoid interferance dermatitis wherein histiocytes were a major cellular component (Fig. 2). Pigmentary incontinence with the presence of melanin, which was engulfed by macrophages beneath *lamina basalis* and a decreased number of epidermal melanocytes were also evident (Fig. 3). Direct immunofluorecsence staining with anticanine IgG and IgA failed to reveal any deposition of immunoglobulins or complement.

Diagnosis

The diagnosis of idiopathic, non-traumatic uveitis with mucocutaneous depigmentation or a Vogt-Koyanagy-Harada-like syndrome was based on the clinical, histological and immunological observations as reported by other authors (1, 8)

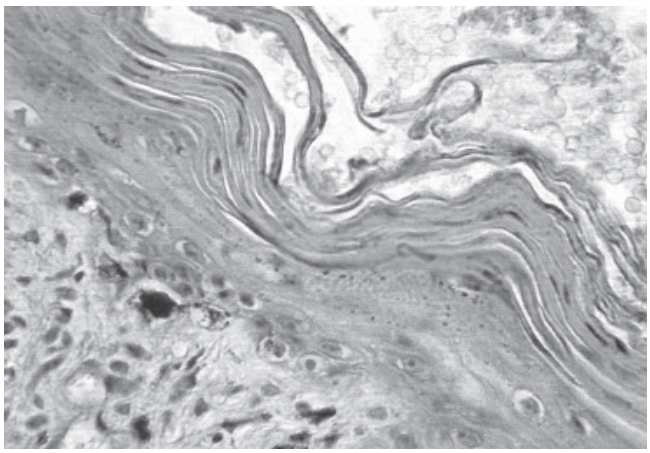


Fig. 2. Irregular epidermal hyperplasia with orthokeratotic hyperkeratosis (HE 80 ×)

depigmentation in nontapetal part of the left eye. Cataract precluded reliable fundoscopy in the right eye. The standard Schirmer test was 18 ± 5 mm OU. To demonstrate the integrity of the corneal epithelium fluorescein was used accompanied with rose bengal staining tests which both proved to be negative. Intraocular pressure was 28 mm Hg OD and 32 mm Hg OS (Schiotz tonometry). Examination of the conjunctival smears showed a normal ocular flora in both eyes. Only rare macrophages and some neutrophils were found.

Skin examination

The dog was clinicaly normal except for abnormalities on physical examination including erythema, depigmentation and ulceration of the nasal planum, lips, scrotum and foot pads. The labial mucosa and soft palate were also ulcerated. Physical examination did not reveal other non-dermatologic systemic diseases.

Complete blood counts, serum panels and urinalyses were normal.

Swab smears from erosions on the nasal planum showed a presence of neutrophils, few macrophages and *Staphylococcus epidermidis*. Permission was not given for ocular biopsies but the owner agreed to skin biopsies. Multiple skin biopsy samples

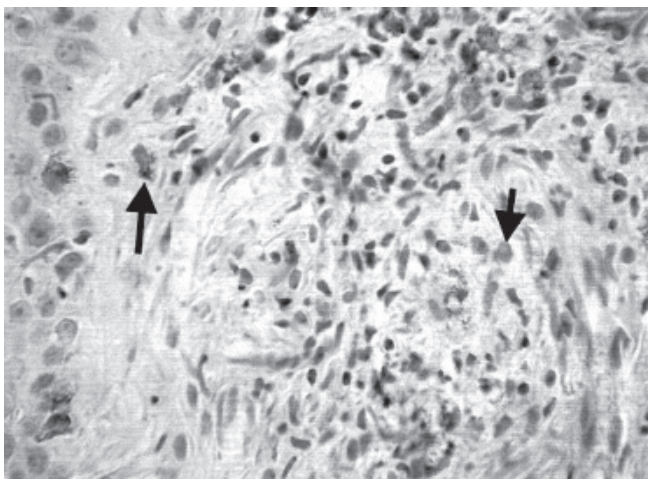


Fig. 3. Lichenoid interface dermatitis with histiocytes (small arrow) and pigmentary incontinence (arrow) (HE 80 ×)

i.e.: A, The simultaneous presence of idiopatic, non-traumatic uveitis and skin lesions. B, Lichenoid infiltration in the upper part of the dermis and pigmentary incontinence. C, The negative results of the direct immunofluorescence test.

Treatment

Therapy for uveitis included topical 1 % Atropin gtt. (UNIMED, Slovak Republic). One drop every two hours was administrated for five days and then three times daily for a week. One drop of 0.2 % Dexamethasone (PHARMACEUTICA, Poland) was given eight times a day for the first week and then was reduced to six times daily on the second week. Systemic therapy included oral Prednisolon tablets (LÉČIVA, Czech Republic) in dose 2 mg per kg^{-1} twice daily for two weeks. Due to the loss of vision the owner decided to have the dog put down in the second week of treatment.

DISCUSSION

The diagnosis of non-traumatic, idiopatic *uveitis with mucocutaneous depigmentation* (Vogh-Koyanagi-Harada-like syndrome) was based on the clinical, histological and immunological examinations.

This clinical case of VKH-like syndrome is interesting with regard to its unique occurrence in the Rottweiler breed. To our knowledge, it has not been previously described in veterinary literature. Even though this syndrome occurs in other breeds it is most frequent in the Akita, the Samoyed and Siberian husky.

From the point of view of differential diagnosis, it is necessary to exclude it from other autoimmune diseases such a *pemphigus* complex and discoid *lupus*, which have distinct histological patterns and typically give positive results in direct immunofluorescence tests. The *uveitis* must be distinguished from other type of inflammatory reactions such as viral, bacterial, and from traumatic etiology.

VKH syndrome is relatively frequent in certain human populations. In Japan it is a cause of 8 % of endogenous uveitis (14). In humans three types of the disease are recognized: 1. *meningoencephalitis*, 2. ocular form and 3. dermatologic form with *alopecia*, *poliosis* and *vitiligo in cervical and thoracic areas*. It is rare for all three forms to be present in one human patient. Ocular and dermatologic forms are most frequent in both human and veterinary medicine. In dogs, a meningoencephalic form of VKH-like syndrome has been up to now described only in one case in Japanese, an Akita which showed meningitis and dysacussis (4).

Moderate to severe anterior or posterior uveitis occurs in humans as well as in dogs whereas in humans it occurs predominantly at a younger age (14). Facial *vitiligo* and *poliosis* appear with a chronic onset of the disease. *Alopecia* and *poliosis* occurs in 90 % of affected humans within three weeks to six years (12). Vitiligo can occur in 50 % of people. These pigmentary changes in humans are permanent with occasional repigmentation (12). Our patient also suffered from *vitiligo*.

Acute or chronic visual loss in humans is relatively frequent; only approximately 30 % of humans affected with VKH syndrome has normal visual acuity. Uveitis in humans is granulomatous, rarely non-granulomatous (16). VKH-like syndrome in dogs must always be considered a severe disease which, if not treated, can have disastrous consequences. Even with therapy and temporary improvement, relapses are frequent (11, 8). We could not observe the results of our intensified therapy because the owner did not consent to further treatment and follow-up of the animal.

CONCLUSION

VHK-like syndrom in dogs is a serious condition. In cases of late or no therapy at all the dogs lose their sight. It needs to be pointed out, however, that relapses occur often even during therapy.

Our case of VKH-like syndrome and others in the veterinary literature, show the importance of early diagnosis and correct therapy to prevent secondary vision-threatening changes which are the reason for the euthanasia of affected animals. To the best of our knowledge, this is one of the first description of VKH-like syndrome in a male Rottweiler dog.

REFERENCES

1. Asakura, S., Takashi. K., Onishi, T., 1977: Voght-Koyanagi-Harada syndrome *(Uveitis diffusa acuta)* in the dog. *Jap. Vet. Med., 673, 445—455.*

2. Bussanich, M. N., Rootman, J., Dolman, C. L., 1982: Granulomatous panuveitis and dermal depigmentation in dogs. *J. Amer. Anim. Hosp. Ass.,* 18, 131—138.

3. Campebell, K. L., McLaughlin, S. A., Reynolds, H. A., 1986: Generalized leucoderma and poliosis following uveitis in a dog. *J. Amer. Anim. Hosp. Ass.,* 22, 121—124.

4. Cottrell, K. L., Barnett, K. C., 1987: Harada's disease in the Japanese Akita. *J. Small Anim. Pract.,* 28, 517—521.

5. Fabries, L., 1984: Syndrome "VKH" chez le chien. Au sujet de deux cas cliniques. *Prat. Méd. Chir. Anim. Comp.,* 19, 393—397.

6. Harada, E., 1926: Clinical study of nonsuppurative chorioiditis. A report of diffuse acute chorioiditis. *Acta Soc. Ophthal. Jap., 30, 356.*

7. Herrera, H. D., Duchene, A. G., 1998: Uveodermatological syndrome (Vogt-Koyanagi-Harada-like syndrome) with generalized depigmentation in a Daschhund. *Vet. Ophthal.,* 1, 47—51.

8. Kern, T. J., Walton, D. K., Riis, R. C., Manning, T. O., Latara, L. J., Dziezyc, J., 1985: Uveitis associated with poliosis and vitiligo in six dogs. *J. Amer. Vet. Med. Ass.,* 187, 408—414.

9. Koyanagi, Y., 1929: Dysakusis, Alopecia und Poliosis bei schwerer Uveitis nicht traumatischen Ursprungs. *Klinische Monatsblatt Augenheilkunde,* 82, 194—211.

10. Madany, J., 2003: Cataract in juvenile dogs-some aspects of clinical examination. *Medycyna Weterynaryjna.*, 59, 70—73.

11. Morgan, R. V., 1989: Vogt-Koyanagy-Harada syndrome in humans and dogs. *Comp. Cont. Educ. Prac. Vet., 11, 1211.*

12. Norlund, J. J., Albert, D., Forget, B., Lerner, A. B., 1980: Halo nevi the Vogt-Koyanagi-Harada syndrome. Manifestation of vitiligo. *Archives of Dermatogy, 116, 690.*

13. Nordlund, J. J., Taylor, N. T., Albert, M., Wagoner, M. D., Lerner, A. B., 1981: The prevalence of vitiligo in patients with uveitis. *Journal of American Academy of Dermatology, 4, 528.*

14. Pulatti, P., Aleci, C., 1987: Malattia di Vogt-Koyanagy-Harada. *Giornale Italiano di Dermatologia e Venerologia,* 122, 305—308.

15. Romatowski, J., 1985: A uveodermatological syndrome in an Akita dog. *J. Amer. Hosp. Ass.,* 21, 777—780.

16. Snyder, D. A., Tessier, H. H., 1980: Vogt-Koyanagi-Harada syndrome. *Amer. J. Ophthalmol.,* 90, 69—75.

17. Vogt, A., 1906: Frühzeitiges Ergraven der Zillen und Bemerkungen über den sogennanten plötzlichen Eintrit dieser Veränderung. *Klinische Monatsblatt Augenheilkunde,* 14, 228—242.

Received September 12, 2003

FOLIA VETERINARIA, 47, 4: 197—199, 2003

THE INTENSIFICATION OF CATTLE BREEDING AND GENETICALLY DETERMINED MORBIDITY (A Review)

Kusenda, M., Kováč, G., Seidel, H., Novotný, J.

**University of Veterinary Medicine, Komenského 73, 041 81 Košice
The Slovak Republic**

SUMMARY

The intensive breeding of cattle and widespread use of artificial insemination significantly increases the risk of the transmission of genetic diseases. Metabolic diseases of genetic origin are characterised by an enzyme deficiency which leads to the accumulation of the substrate or an alternative metabolic pathway. Hereditary transmission is of an autosomal recessive type. The variable clinical signs require the use of laboratory tests for diagnosis.

Key words: cattle; genetic; metabolic disease

INTRODUCTION

The health management and protection of animals with a subsequent production of safe food of animal origin depend directly on rearing conditions (2). In the prevention of genetic disorders, special approaches are required. In this work we present the basic data on the causes, occurrence, clinical signs and diagnosis of metabolic disorders of genetic origin. The data from 1990 to 2000 are summarised in Table 1.

CAUSES, PHENOTYPIC EXPRESSION, AND PATOPHYSIOLOGY

The primary cause of the disorders mentioned above is usually a point mutation in the enzyme coding gene. The pattern of inheritance is monogenic autosomal recessive and follow Mendelian rules. Therefore, heterozygotes are unaffected phenotypically. They represent a latent carrier status, that is responsible for the spread of such diseases in the population. The latent carrier frequencies are many times more numerous as well as harmful than individuals affected. Only recessive homozygotes are affected (both alleles mutant) with an equal probability in both sexes. Gene mutation results in stopping or strongly reducing the synthesis of specific enzymes (6). The subsequent substrate accumulation or changed metabolic pathway determines the different mechanisms of the disease:

1. Accumulation of the substrate

Non-degraded substrate of various chemical compositions (glycide, glycoprotein, glycolipid, lipoprotein) is accumulated in lysosomes followed by their hypertrophy (lysosomal storage disease).

2. Toxicity of the non-degraded substrate

A deficiency in argininosuccinate synthetase (enzyme of the urea cycle), by citrullinaemia, leads to the accumulation of neurotoxic ammonia in the brain which is the cause of death in affected calves (7).

3. Deficiency of essential components

With a deficiency of uridine monophosphate synthase (DUMPS), the blockage of orotic acid conversion to uridine monophosphate (essential part of pyrimidine bases) causes death of the embryo before the 40th day, when embryonic stores are exhausted and transplacentar transfer is impossible (5).

4. Metabolic reorientation

When substrate accumulation is not dominant, the metabolic pathway acquires a new orientation. This is the case of erythropoietic porphyria, when porphobilinogen is directed to the synthesis of serial isomers (1).

Table 1. Metabolic diseases of genetic origin (1, 3, 4, 5, 6, 7)

Name	Enzyme *substrate	Breed *age	Clinical signs	Pathological signs	Diagnosis
Ehlers-Danlos syndrome	Procollagen peptidase *procollagen	Holstein Simmental Charolais Hereford	fragile skin lacerations oedema of the legs and eyelids	abnormal collagen in the skin	histology of the skin
Deficiency of uridine monophosphate synthase (DUMPS)	uridine monophosphate synthase *orotic acid	Holstein *embryo	embryonic death		concentration of orotic acid in urine UMPS activity in embryocytes, PCR
Maple syrup urine disease	alpha-ketoacid dehydrogenase complex *alpha-ketoacids	Hereford, Angus *0—5 days	hyperthermy apathy static ataxia, tremor hyperaesthesia, rigidity of muscles maple syrup urine	vacuolar myelinopathy without neuronal and glial damage (cerebrum, cerebellum spinal cord)	plasma, serum tissues: complex amino-acids
Citrullinaemia	argininosuccinate synthetase *citrulline	Holstein, *0—4 days	hyperthermy, depression, blindness spasms, coma (ammonia poisoning)	oedema of the brain neuronal vacuolisation, steatosis	plasma, serum: citrulline, ammonia PCR
Glycogenosis type II (Pompe disease)	alpha-glucosidase *glycogen	Brahman Shorthorn *3—6 months	poor growth incoordination muscle weakeness recumbency, paralysis of the tongue	multifocal vacuolisation: brain (neurons) muscles (sceletal, myocard, digestive tract), kidneys (tubules), liver (hepatocytes), PAS +	Alpha-glucosidase activity, histology: brain, muscles, cytochemistry: PAS + in lymphocytes
GM 1 gangliosidosis	GM 1 β-galaktosidase *GM 1 gangliosid	Frisonne, *2 weeks – 3 months	progressive ataxia difficult rumination and swallowing blindness, tetraplegia progressive kachexia	neurons: lysosomal storage of gangliosid cytoplasmatic granules: PAS +, black sudan -, blue luxol +	histology of the nervous system β-galactosidase activity in leukocytes
Ceroid lipofuscinosis (Batten's disease)	Unknown *C subunit of mitochondrial ATP synthase	Devon *6—18 months	blindness (central, later peripheric), symetric tremor of muscles depression, spasms	cerebral atrophia, neurons, myocytes, hepatocytes, Kuppfer's cells, tubular cells kidneys), pancreas: storage of cytosom (PAS, black sudan, eosine, Ziehl)	histology of nervous system fluorescency
α-mannosidosis	α-mannosidase *oligosaacharides	Angus, Gallovay Murray gray *from 1 day to few months	abortion, progressive ataxia (static and kinetic), tremor, agresivity, poor growth	cytoplasmatic vacuoles (neurons, endothelium, macrophags, epithelium — pankreas, kidneys)	histology of the brain, α-mannosidase activity (serum, plasma, leukocytes)
β-mannosidosis	β-mannosidase *oligosacharides	Salers *from the birth	head malformation brachygnatia superior reduced moving of the eyelids, tremor, nystagmus	hypertrophy of kidneys and thyroid gland, hydrocephalia, cytoplasmatic vacuolisation (neurons, macrophags, epithelium — kidneys, thyroid gland)	histology (brain, thyroid gland, thymus, pancreas, kidneys), β-mannosidase activity (plasma lymphocytes)
erythropoietic protoporphyria	Ferrochelatase *protoporphyrine IX	Limusine, Aquitaine *from few days to months	poor growth, epilepthiform crises photosensibilisation	pigmentation of the liver in porthal area	porphyrins (blood) ferrochelatase activity in leukocytes
erythropoietic porphyria	Uroporphyrinogen III cosynthetase *uroporphyrin and coporphyrin type I	Holstein, Ayshire	brown pigmentation of the teeths and bones, brown urine haemolytic regenerative anaemia, photosensibilisation	brown pigmentation of the teeths and bones	fluorescency (red-orange) blood porphyrine

DIAGNOSTICS AND DETECTION

Clinical examination reveals only a diagnosis of a suspected genetically conditioned metabolic disorder. The similarity of clinical signs to other (both infectious and non-infectious) diseases are indicative for laboratory tests. These tests use histopathology, specific staining, and excretion of non-degraded substrates (e. g. in urine).

The determination of enzymatic activities (plasma, tissues, leukocytes) can be also used in herd surveys and the detection of heterozygous carriers — enzymatic activity is lower (theoretically by 50 %) than in homozygotes, for example in mannosidoses (6) or DUMPS (4).

Recently, molecular-genettic methods (PCR — polymerase chain reaction, restriction enzymes, etc.) have been successfully used with direct analysis of DNA. The advantage of this gene-typing is accuracy in identifying mutated allele in heterozygotes.

CONCLUSION

The peculiarity of aetiology and transmission of genetic diseases in veterinary medicine requires, first of all, sound prevention. Therefore, the detection of carriers (particularly breeding animals) is especially important to protect herds against the occurrence and spread of these diseases.

ACKNOWLEDGMENT

This work was supported by VEGA: Grant No. 1/7026/20.

REFERENCES

1. Franco, D. A., Lin, T. L., Leder, J. A., 1992: Bovine congenital erythropoietic porphyria. *Comp. Cont. Educ. Food Anim.,* 14, 822—826.

2. Kováč, G., 2000: Current health problems and their possible solution in cattle breeding. *Folia Vet.,* 44, 158—161.

3. Kováč, G. *et al.,* 2001: *Diseases of Cattle* (In Slovak). Publ. House M & M Prešov (SR). 2001, 874 pp.

4. Shanks, R. D., Robinson, J. L., 1990: Deficiency of uridine monophosphate synthase among Holstein cattle. *Cornell Vet.,* 80, 119—122.

5. Shanks, R. D., Robinson, J. L., 1989: Embryonic mortality attributed to inherited deficiency for uridine monophosphate synthase. *J. Dairy Sci.,* 72, 3035—3039.

6. Schelcher, F., Valarcher, J. F., Foucras, G., Espinasse, J., 1995: Maladies métaboliques d' origine génétique. *Point. Vét.,* 27, 801—806.

7. Thornton, R. N., Gilmour, M. L., Rammel, C. A., 1991: Citrullinaemia in Friesian calves. *N. Z. Vet. J.,* 39, 145—146.

Received May 20, 2003

FOLIA VETERINARIA, 47, 4: 200—203, 2003

A LIGHT-MICROSCOPIC STUDY OF AN EARLY PHASE OF PARAPLEGIA INDUCED BY ISCHEMIA AND REPERFUSION IN DOGS

Šulla, I.[1], Maršala, J.[2], Lukáč, I.[3]

[1]University of Veterinary Medicine, Komenského 73, 040 81 Košice
[2]Neurobiological Institute of the Slovak Academy of Sciences Šoltésovej 4, 040 01 Košice
[3]Department of Neurosurgery, University Hospital, Tr. SNP 1, 040 66 Košice
The Slovak Republic

sulla@uvm.sk

ABSTRACT

Several aspects of pathophysiologic mechanisms involved in the development of paraplegia induced by ischemia and reperfusion are only poorly understood and their histologic manifestation still remains incompletely documented. This inspired the authors to study light-microscopic changes of spinal cord neurons caused by a temporary high thoracic aorta occlusion and reperfusion. Nine adult mongrel dogs, weighing eighteen to twenty-five kilograms, were used in the study. Three animals served as sham controls, in six the thoracic aorta was constricted by a tourniquet applied on the vessel through a thoracotomy for thirty minutes, which was followed by thirty minutes of survival. The procedures were performed in general anaesthesia induced by pentobarbital (30 mg.kg^{-1} i.v.), maintained with mixture of 1—2 % halothane with oxygen. Experiments were finished by perfusion (3000 cc of saline) and fixation (3000 cc of 10 % neutral formaldehyde) of all animals, then 30 µm thick sections from L3–S1 spinal cord segments were processed by the Nauta staining method. Light-microscopic changes in experimental animals were characterized by distinct argyrophilia of neurons in all but three superficial dorsal horn layers, of interneurons in *zona intermedia*, and motoneurons in ventral horn layers. Since normal neural cells are Nauta-negative, their silver impregnation after thirty minutes of ischemia, followed by thirty minutes of reperfusion, can be considered a histopathologic manifestation of an early phase of neuronal damage, leading, without treatment, to cell death and irreversible paraplegia.

Key words: histopathology; ischemia-reperfusion; neuronal damage; paraplegia

INTRODUCTION

The potential development of neurological sequelae after the temporary occlusion of the thoracic aorta has been recognized by cardiovascular surgeons for a long time (1, 3, 9, 16). Despite the evolution of a number of management strategies and the use of different drugs with the aim of avoiding the development of postoperative paraplegia, even in the most experienced hands the risk of this devastating complication is currently approximately 10 % (1—3, 6, 9—11, 17). Early signs of ischemically induced neuronal damage leading subsequently to cell death with all neurological consequences remain unclear and discrete neuropathological changes related to this nosological entity are incompletely documented (4—7, 14). That is why we decided to carry out a light-microscopic study of an early phase of paraplegia induced by ischemia and reperfusion in a canine experimental model.

MATERIALS AND METHODS

The experimental protocols were elaborated in compliance with the *Animal Protection Act of the Slovak Republic No. 15/1995 and approved by the Ethical Commission of the Neurobiological Institute of the Slovak Academy of Sciences in Košice.*

Nine adult mongrel dogs of both sexes, free of heart worm disease, weighing eighteen to twenty-five kilograms were used in the study. Animals were anaesthetized with pentobarbital *(Pentobarbitalum natricum* — "Pentobarbital", SPOFA, Prague) administered intravenously in a 30 mg.kg^{-1} dose, then intubated with an endotracheal cannula ("Portex", BERCK, Paris) of a diameter 8—12 mm, and placed on a volume-cycled ventilator ("Anemat N 8", CHIRANA, Stará Turá, SR). The anaesthesia was maintained by a mixture of medical oxygen with 1—2 %

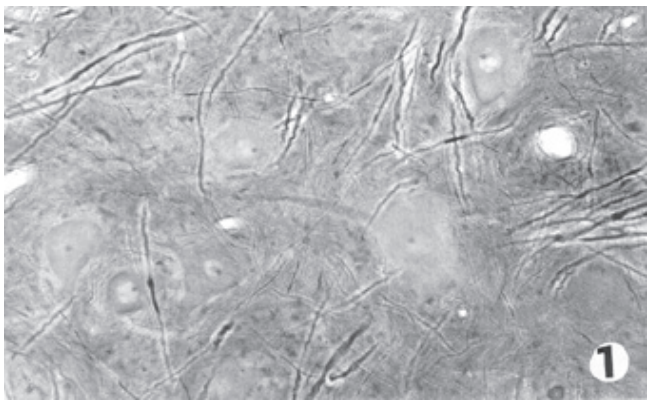


Fig. 1. Light-microphotograph of control section from L 5 spinal cord segment. Large motoneurons with darker nucleoles are Nanta-negative and appear as pale shadows almost identical with neuropil (× 180)

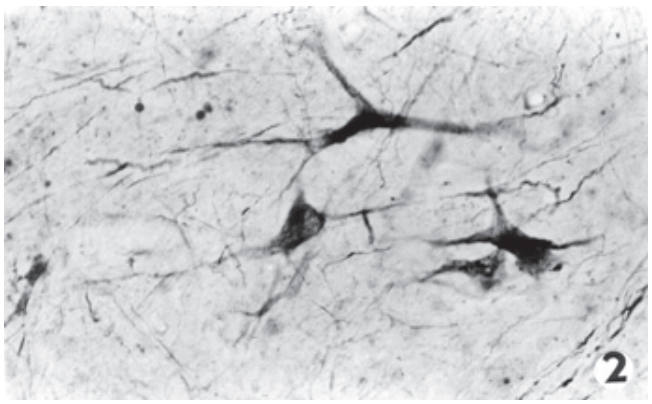


Fig. 2. Light-microphotograph of the L 5 spinal cord segment after thirty minutes of ischemia followed by thirty minutes of reperfusion. Several middle-sized impregnated neurons with intracytoplasmic dark particles in the basal portion of the posterior horn (× 120)

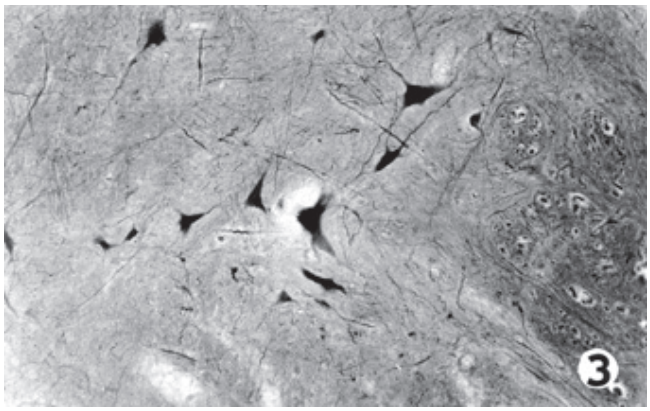


Fig. 3. Light-microphotograph of the L 5 spinal cord segment after thirty minutes of ischemia followed by thirty minutes of reperfusion. Heavily impregnated interneurons of Rexed's lamina VII (× 120)

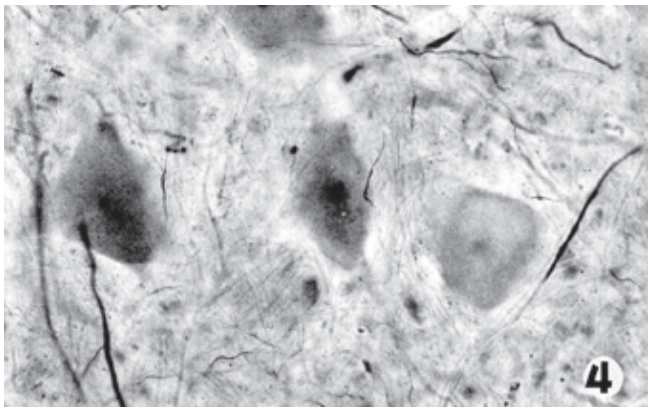


Fig. 4. Light-microphotograph of the L 5 spinal cord segment after thirty minutes of ischemia and thirty minutes of reperfusion. Lightly and irregulary impregnated motoneurons in the ventromedial part of anterior spinal cord horn (× 180)

narcotan (*Halothanum thymolum stabilisatum* — "Narcotan", LÉČIVA, Prague). The continuous direct monitoring of the arterial blood pressure in a radial artery (monitor LMP 150, TESLA, Piešťany, SR), EKG (monitor LKM 220, CHIRANA, Stará Turá), and arterial blood gases (Automatic Gas Check 995 Hb, CMI, Wien) were performed. The rate of ventilation was adjusted to maintain arterial pO_2 between 80—100 mm Hg and pCO_2 at about 38 mm Hg (normal canine levels). The arterial blood pressure (which increased in each dog after the high thoracic aorta occlusion) was held on at presurgical levels by an infusion of nitroprusside (*Natrii nitroprussidum* dihydricum — "Nipride", ROCHE, Paris).

The dogs were divided into two groups:

1. Non-ischemic controls (n = 3)

The animals underwent a left-sided thoracotomy through the fifth intercostal space. After sixty minutes of survival they were transcardially perfused with 3000 cc of saline and fixed by the same volume of 10 % neutral formaldehyde.

2. Ischemia-reperfusion injuries of the spinal cord (n = 6).

The descending aorta, just distal to the origin of the left subclavian artery, was occluded by tourniquet for thirty minutes, then the vessel was released and after thirty minutes of survival (reperfusion period) the animals were sacrificed by the same method as non-ischemic controls.

The *spinal cords* of all animals were removed in *toto* and at twenty-four hours postfixed with formaldehyde. Specimens comprising L3—S1 spinal cord segments were cut by microtome in semithick (30 μm) sections and processed according to the Nauta staining method for light-microscopic observations (7, 8, 10).

RESULTS

The identity of the neurons in semi-thick sections of the L3—S1 spinal cord segments of control animals processed according to the Nauta method was hardly distinguishable. The perikarya were only minimally impregnated and their dendritic ramifications did not stain at all. Lightly Nauta-positive cytoplasm of middle-sized and large neural cells (especially the large motoneurons in ventrolateral parts of anterior gray matter horns) appeared as pale shadows, almost identical with the background. The localisation of neural cell somata was facilitated by large light nuclei and darkly stained nucleoles (Fig. 1).

The neuronal damage of the L3—S1 spinal cord segments after thirty minutes of ischemia followed by

thirty minutes of reperfusion was characterized by the occurrence of many clearly impregnated argyrophilic somata and dendrites representing small and middle-sized interneurons localized in Rexed's laminae V—VII. Some neurons were shrunk and their Nauta positivity was accentuated due to the occurrence of dark intracytoplasmatic particles (Fig. 2).

The highest sensitivity to ischemic-reperfusional injury was observed in a group of small neurons (< 15 μm) localized in Rexed's laminae V—VII, where neurohistologic changes were characterized by marked argyrophilia of dendrits and somata of shrunken, and deformed cells (Fig. 3).

Less profound and quite irregular changes were observed in a group of large motoneurons of ventral spinal cord horn-neurons were of irregular shape, but not shrunk or disintegrated; majority of them contained argyrophilic intracytoplasmic particles, however there were nuclei in some of them, as well (Fig. 4).

DISCUSSION

The term paraplegia refers in mammals to the permanent loss of voluntary control of the hind limb and tail muscles, anal and urinary bladder sphincters, the disturbance of sensitivity and sexual incompetence (7, 10).

A very similar neurological deficit is connected with spinal cord damage in humans. The main cause of its development is trauma to the backbone and its neural structures. In the second place is a temporary or permanent interruption of spinal cord blood flow. To awake from anaesthesia after an operation on the aorta with fully developed paraplegia means a catastrophe for both, the patient and the cardiovascular surgeon as well (9, 14, 16). Various methods have been used in attempts to decrease the incidence of this complication (e.g. the abbreviation of an ischemic period, hypothermia, epidural cooling, cerebrospinal fluid drainage, application of corticosteroids, oxygen radical scavengers administration and a number of other pharmacological interventions), however with varying degrees of success (1—3, 5—7, 9—11, 15—18). The reason is a limited knowlege of the initial events triggered in the brain or spinal cord neurons by ischemia and reperfusion. Potentially they could be reversed during this early postischemic phase, but without immediate application of appropriate therapeutic measures the destructive metabolic cascade continues until it ends in an apoptotic cell death (4, 7, 10, 13).

The results of our study concur with numerous clinical and experimental observations (2, 7, 9, 13, 16—18). They confirm the rapid development of distinct neuronal changes in spinal cord neurons of dogs induced by ischemia and reperfusion (eventually leading to fully developped paraplegia) and show why curative interventions fail when delayed (2, 11, 14, 16). This is a valuable concept in this study, explaining the clinical observation, that therapeutic window after spinal cord ischemia is short (2).

The canine experimental model used in the study was elaborated with the aim of being reproducible, to imitate the clinical situation during aortic surgery in humans and inevitably causes ischemic, and ischemia-reperfusional damage to the spinal cord in dogs (10, 12). Studies at seventy-two hours of survival showed that our technique was highly effective — all experimental animals awakened from anaesthesia with fully developed paraplegia (12).

CONCLUSIONS

Severe neurologic deficit remains a potentially devastating complication of aortic surgery. The time interval required for development of ischemic-reperfusional spinal cord damage is short. The support of spinal cord blood flow and diminishment of neural tissue vulnerability are the best available therapeutic measures, so far. Further research of metabolic processes leading to neuronal damage is necessary.

REFERENCES

1. Ackerman, L. L., Traynelis, V. C., 2002: Treatment of delayed-onset neurological deficit after aortic surgery with lumbar cerebrospinal fluid drainage. *Neurosurgery,* 51, 1414—1422.

2. Albin, M. S., White, R. J., 2000: Therapeutic window after spinal cord trauma is longer than after spinal cord ischemia. *Anaesthesiology,* 92, 281—282.

3. Cunningham, J. N., jr., 1998: Spinal cord ischemia: introduction. *Sem. Thoracic Cardiovasc. Surg.,* 10, 3—5.

4. Kanellopoulos, G. K., Kato, H., Hsu, C. Y., Kouchoukos, N. T., 1997: Spinal cord ischemic injury. Development of a new model in rat. *Stroke,* 28, 2532—2538.

5. Kato, N., Yanaka, K., Nagase, S., Hirayama, A., Nose, T., 2003: The antioxydant EPC-K1 ameliorates brain injury by inhibiting lipid peroxydation in a rat model of transient focal cerebral ischemia. *Acta Neurochir.* (Wien), 145, 489—493.

6. Lang-Lardunski, L., Heurteaux, C., Dupont, H., Rouelle, D., Widmann, C., Matz, J., 2001: The effects of FK 506 on neurologic and histopathologic outcome after transient spinal cord ischemia induced by aortic cross-clamping in rats. *Anaesth. Analg.,* 92, 1237—1244.

7. Maršala, M., Vanický, I., Yaksh, T. L., 1994: Effect of graded hypothermia (27 to 34 degrees C) on behavioral function, histopathology, and spinal blood flow after spinal ischemia in rat. *Stroke,* 25, 2038—2046.

8. Nauta, W. J. H., 1957: Silver impregnation of degenerating axons. In **Windle, W. P.** (ed.): *New Research Techniques of Neuroanatomy.* Charles C. Thomas Publ., Springfield Il., 17—26.

9. Pecháň, I., Záhorec, R., Holomáň, M., Plvanová, A., 1996: Protective effect of antioxydative vitamins and creatinphosphate in patients during cardiosurgical operations (In Slovak). *Slov. Lekár,* 6, 16—19.

10. Radoňák, J., Maršala, M., Maršala, J., 1999: Graded postischemic reoxygenation as a measure of spinal cord protection in experiments (In Slovak). *Rozhl. Chir.,* 78, 105—108.

11. Robertazzi, R. R., Cunningham, J. N., jr., 1998: Intraoperative adjuncts of spinal cord protection. *Sem. Thoracic Cardiovasc. Surg.,* 10, 29—34.

12. Šulla, I., jr., Maršala, J., Šulla, I., 2003: Study of ischemic and compressive paraplegia in dogs. *Proceedings of the 12th European Congress of Neurosurgery,* Lisboa, Portugal, 322.

13. Taoka, Y., Okajima, K., 1998: Spinal cord injury in the rat. *Prog. Neurobiol.,* 56, 341—358.

14. Vajó, J., 1996: *Sudden Angiosurgical Events and Acute Ischemic-reperfusional Syndrome in Clinical Practice* (In Slovak). P. J. Šafárik University Publishing Center, Košice, 148 pp.

15. Vanický, I., Urdzíková, L., Saganová, K., Maršala, M., 2002: Intrathecal methylprednisolone does not improve outcome after severe spinal cord injury in the rat. *Neurosc. Res. Comm.,* 31, 183—191.

16. Webb, T. H., Williams, G. M., 1999: Thoracoabdominal aneurysm repair. *Cardiovasc. Surg.,* 7, 573—585.

17. Zhang, P., Abraham, V. S., Kraft, K. R., Rabchevsky, A. G., Scheff, S. W., Swain, J. A., 2000: Hyperthermic preconditioning protects against spinal cord ischemic injury. *Ann. Thoracic Surg.,* 70, 1490—1495.

18. Zhao, B. Q., Suzuki, Y., Kondo, K., Ikeda, Y., Unemura, K., 2001: Combination of a free radical scavenger and heparin reduces cerebral hemorrhage after heparin treatment in a rabbit middle cerebral artery occlusion model. *Stroke, 32, 2157—2163.*

Received September 10, 2003

FOLIA VETERINARIA, 47, 4: 204—206, 2003

THE EFFECT OF AMPHETAMINE ON THE DEVELOPMENT OF OEDEMA DISEASE IN PIGS

Horn, C. R., Bilkei-Papp, G. M.

Veterinary Consulting, Raubbühlstrasse 4, CH – 8600 Dübendorf Switzerland

ABSTRACT

The objective of this trial was to determine if the oral application of amphetamine would hinder the development of oedema disease in pigs, after an intravenous challenge with *Escherichia coli* verotoxin (VT2e toxin). Two randomised treatment groups of weaned piglets were formed. The animals in group one (n = 30) were intravenously challenged with 25 µg of VT2e toxin. After the first signs of palpebral oedema, the weaners were treated with 1 mg.kg^{-1} b. w. *amphetaminum hydrochloricum*. Group two (n = 30) were intravenously challenged with 25 µg of VT2-e toxin as well; these animals received no further treatment. Diarrhoea, respiratory distress, ataxia, incoordination were significantly (P < 0.05) milder in the amphetamine-treated than in untreated animals. Fifteen of the thirty piglets died within four days in the untreated group, but only two pigs died in the amphetamine-treated group of pigs (P < 0.05). At necropsy, significant (P < 0.05) differences were found regarding the excess amount of cerebrospinal fluid, fibrinous peritonitis and fibrinous pleuritis among the amphetamine-treated than in untreated animals. Microscopic examination of the brain revealed a higher degree of cerebrospinal angiopathy in the untreated dead animals. The implication is that amphetamine administered at an early stage of VT2e toxin-caused clinical signs prevents the development of oedema disease in swine.

Key words: amphetamine; oedema disease; piglets; verotoxin; weaning

INTRODUCTION

"Postweaning colibacillosis" is one of the major causes of death, reduced health and unsatisfactory economic performance in recently weaned pigs. From the practical point of view post-weaning (PW) *Escherichia (E.) coli* has caused problems ("post-weaning coli complex", PWCC) — Bilkei *et al*. (2) can be subcategorised into the following manifestations: post-weaning diarrhoea (PWD), oedema disease (ED), post-weaning wasting (PWW) and haemorrhagic gastroenteritis (HGE) (5). Since the introduction of intensive pig production, PWCC-caused losses has markedly increased (4). ED occurs usually in the first two weeks after weaning (1). In western Europe the disease is often caused by haemolytic F18 pilus-positive *E. coli* O139 and O141 that produces verotoxin 2-e (8). VT2e toxin injected in pigs reproduces the clinical and pathological signs of ED (7). The therapy of ED is controversially discussed in the literature (1, 5, 3, 4).

Verotoxigenic *E. coli* colonise the intestine via F18 pilus (12). Pigs suffering from a chronic, subclinical form of ED do not suffer mortality, but develop progressive vascular necrosis and PWW, causing enormous economic losses (3). If *E. coli* O139 has the ability to produce a heat-labile toxin (LT), heat-stable toxins (STa, STb), enterotoxin (VT2e), both PWD and ED can develop (2).

Control of PWCC (especially ED and PWW) includes:

- Immunoprophylaxis using verotoxin-toxoids (1).
- Improving social and environmental conditions at weaning (3).
- Nutrition of the weaned pigs (3).
- PW zoo- and biotechnique, including the application of antibiotics, probiotics, tripellenamin, melperone (9), amperozide, clorpromazin, central nervous stimulants (10), prednisolone or zink-oxyde and phytogenic feed additives (5, 2, 3, 4).

The objective of the present trial was to determine the effect of amphetamine on the development of oedema disease in piglets after challenge with VT2e toxin.

MATERIAL AND METHODS

In a large German pig production unit seven weaned litters (60 piglets, 28 ± 8 days of age, 8.01 ± 0.74 kg b. w.) were selected for the study. The herd was free from *Actinobacillus pleuropneumoniae*, toxin producing *Pasteurella multocida* and

Brachyspira hyodysenteriae. In this unit *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis* were endemically present, causing no mortality but a decreased post-weaning performance, such as low weight gain, suppressed growth and feed efficiency.

Two randomised (computer-generated list within blocks of similar weights in a 1:1 assignment ratio) parallel treatment groups of piglets were formed. The weaners were ear tagged according to group and relocated in large nursery barns, in pens (ten piglets per pen). The building was a totally confined and hygienically controlled facility (22—24 °C, air movement 0.1—0.2 m.sec^{-1}, average relative humidity 72—77 %, 0.5 m^2 floor per pig). The floor of the pens were 2/3 slatted concrete over a shallow pit. A stainless steel self-feeder and automatic nipple waterer serviced each pen.

The groups were treated as follows: Group one (n = 30): 25 µg of VT2-e toxin were given intravenously. After the first signs of palpebral oedema (six hours after VT2-e toxin application) the weaners were treated with 1 mg.kg^{-1} b. w. *amphetaminum hydrochloricum,* given intragastrically via a stomach tube. Group two (n = 30), 25 mg of VT2-e toxin were given intravenously. The animals received no further treatment.

The following parameters were evaluated:

Clinical evaluations: Piglets were examined for four days, five times daily, for ED typical clinical signs (Table 1).

Mortality was recorded.

All remaining piglets were put down (euthanasia was adminstered) on Day 4 and a gross pathological examination of the carcases and microscopic examination of their brains were performed (Table 1).

GLMM (general linear mathematical model) and REML (reference manual) procedures of the statistical package of Genstat 5, Oxford, Clarendon and Fisher's exact test were used to analyse data. For time aspects (such as the exact age of the piglets at the time of toxin application) and proportion of piglets dying for ED a logistic regression model was used. The effect of the sow and the interaction between treatment and sow were included as random components, but had no significant influence on mortality due to ED.

RESULTS AND DISCUSSION

The majority of clinical signs, such as diarrhoea, laboured breathing, dyspnea, ataxia, lack of coordination were significantly ($P<0.05$) milder in amphetamine-treated than in untreated animals. There were no significant differences between the groups in terms of spasms and mental confusion ($P>0.05$). Fifteen of the thirty piglets died within four days in the untreated group. Two of the thirty died in the amphetamine-treated group of pigs ($P<0.05$) (Table 1).

At necropsy significant ($P<0.05$) differences were found regarding the excess amount of cerebrospinal fluid, fibrinous peritonitis and fibrinous pleuritis between the amphetamine-treated and untreated animals, while oedema in subcutis, lymphnodes, stomach wall and mesenterium revealed no significant differences between the groups ($P>0.05$) (Table 1).

Table 1. The effect of amphetamine on the development of oedema disease in pigs following challenge with Escherichia coli verotoxin-2e

	Group 1 n/N %	Group 2 n/N %	P-value
Clinical signs			
Diarrhoea	4/30 = 13.3	30/30 = 100	0.049
Laboured breathing, dyspnea	3/30 = 10	30/30 = 100	0.048
Spasms	12/30 = 40	20/30 = 77	0.161
Ataxia, incoordination	3/30 = 10	30/30 = 100	0.048
Mental confusion	30/30 = 100	30/30 = 100	1
Mortality	2/30 = 6.7	15/30 = 50	0.049
Macroscopic lesions			
oedema in subcutis	30/30 = 100	30/30 = 100	1
oedema in lymphnodes	13/30 = 43.3	21/30 = 70	0.159
oedema of stomach wall	12/30 = 40	22/30 = 73.3	0.150
mesenteric oedema	14/30 = 46.7	20/30 = 77	0.161
excess amount (>6 ml) of cerebrospinal fluid	3/30 = 10	30/30 = 100	0.048
fibrinous peritonitis	3/30 = 10	30/30 = 100	0.048
fibrinous pleuritis	4/30 = 13.3	30/30 = 100	0.049
Microscopic examination of brain			
Cerebrospinal angiopathy	**3/30 = 10**	**30/30 = 100**	**0.048**

Group one: 25 mg of *Escherichia coli* VT2e toxin were given intravenously. After the first signs of palpebral oedema the weaners were treated with 1 mg.kg^{-1} b. w. *amphetaminum hydrochloricum*

Group two: 25 mg of *Escherichia coli* VT2e toxin given intravenously. The animals received no treatment.

n: positively diagnosed cases **N:** number of piglets in a group

The **Microscopic examination** of the brain revealed in the untreated dead animals a high degree of vascular lesion, demyelination fibrinous or hyaline necrosis of the arterial walls, perivascular emergence of eosinophilic droplets, thrombosis and malacia. Vascular lesions varied in severity, depending on their location in the brain (most severe in the *medulla oblongata,* pons and mid brain). Arterioles and small sized arteries showed hyalin degeneration of the medial cells, often accompanied by a narrowing of the vascular lumen and thrombosis and perivascular emergence of eosinophilic droplets of various sizes. The characteristic lesions in arterioles and small arteries were insudations and necrosis in the media with the endothelial cell layer essentially intact. The arterial endothelial cells showed oedematous swellings and some dissociation of tight junctions. Although similar lesions were found in the treated animals as well, they were more severe in the untreated ones.

ED causes serious losses in pig rearing. Unlike the diarrhoeic *Escherichia coli* infections induced by enterotoxins directly damaging the intestinal mucosa, ED develops after the absorption of VT2-e into the blood. The impairment of the permeability of blood vessels induced by VT2-e toxins results in the development of oedema of subcutaneous tissue, mesenterium and brain. The immediate cause of death in serious ED cases is brain oedema and cerebrospinal angiopathy. Cerebrospinal angiopathy in swine is characterised by lesions in the arterioles, primarily in the brain stem, which include subendothelial hyalin droplets, degeneration or fibrinoid necrosis of the media and perivascular eosinophilic droplets (2).

According to previous publications (1) chemotherapeutic control of bacterial proliferation is effective in PWD, but there is not much chance of saving the lives of piglets in advanced ED cases, showing severe subcutaneous oedema, respiratory distress, ataxia and an inability to rise. Orban *et al.* (10, 11) have published data on successful ED therapy with amphetamine, Olsson and Olsson (9) have reported the therapeutic success of ED with melperone. The repeatability and evaluation of the therapeutic effect of these substances is difficult, because the severity of ED and the speed of impending pathological changes cannot be quantified (2). Amphetamine or melperone, given after cerebrospinal angiopathy has caused changes and the completion of pathological signs in the brain, are of little help (5).

According to Bilkei *et al.* (3, 4) prophylactic application of amphetamine prevents the full manifestation of impending ED. Although these authors have stated that none of the therapeutic methods are able to prevent death due to ED completely, amphetamine elicits its therapeutic effect on the same areas in the central nervous system where cerebrospinal angiopathy produces its major pathological effects, preventing the full manifestation of VT2e toxin caused tissue damage.

REFERENCES

1. Awad-Masalmeh, M., Schuh, M., Kofer, J., Quakyi, E.. 1989: Verification of the protective effects of toxoid vaccine against edema disease of weaned piglets in an infection model. *Dtsch. Tierärztl. Wschr.,* 96, 419—421.

2. Bilkei, G., Biro, O., Bölcskei, A., Clavadetscher, E., Orban, P., Waller, C., 1995: Practice related management strategies on post-weaning *E. coli* problems in the intensive pig production. *Hung. Vet. J.,* 10, 776—777.

3. Bilkei, G., Bölcskei, A., Clavadetscher, E., Biro, O., Takacs, T., Kotai, I., Uys, A., 1996b: Field trials on early stage of postweaning coli complex oedema disease in intensive pig production units. *Proc. 14th IPVS Congress, Bologna, ltaly,* 257.

4. Bilkei, G., Clavadetscher, E., Takacs, T., Bölcskei, A., Kotai, I., Uys, A., 1996a: Comparison of the effect of Zoo- or combined Zoo- and Biotechnique on the postweaning parameters of the piglets. *Proc. 14th IPVS Congress, Bologna, ltaly,* 266.

5. Bölcskei, A. and Bilkei, G., 1994: Therapie der Ödemkrankheit/Colikomplex der Absatzferkel – Wo stehen wir heute? *Berl. Münch. Tierärztl. Wschr.,* 107, 82—85.

6. Bölcskei, A., Bilkei, G., Biro, O., Clavadetscher, E., Goos, Th., Waller, C., and Stelzer, P., 1995: Management der *E. coli*-bedingten Faktorenkrankheiten nach dem Absetzen der Ferkel. *Berl. Münch. Tierärztl. Wschr.,* 109, 108—111.

7. Johansen, M., Andersen, L. O., Jorsal, S. E., Thomsen, L. K., Waddel, T. E., Gyles, C. L.,1997: Prevention of edema disease in pigs by vaccination with verotoxins 2 E Toxoid. *Canadian J. Vet. Res.,* 61, 280—285.

8. Macleod, D. L., Gyles, C. L., 1991: Immunisation of pigs with a purified Shiga-like toxin II variant toxoid. *Vet. Microbiol.,* 29, 309—318.

9. Moon, H. W., Hoffmann, L. J., Cornick, N. A., Boother, S. L., Bosworth, B. T. 1999: Prevalences of some virulence genes among *Escherichia coli* isolates from swine presented to a diagnostic laboratory in Iowa. *J. Vet. Diagn. Invest.,* 11, 557—560.

10. Olsson, T., Olsson, S. O., 1982: Melperone treatment of edema disease of pigs. *Proc. 14th IPVS Congress, Mexico City, Mexico,* 26.

11. Orban, P., Bilkei-Papp, G., Bölcskei, A., 1993: Bericht über die therapeutische Wirkung von zentralnervösen Stimulantien bei der Ödemkrankheit der Schweine. *Berl. Münch. Tierärztl. Wschr.,* 106, 423—425.

12. Orban, P., Bilkei-Papp, G., Waller, C., Bölcskei, A. 1994: Beitrag zur Therapie der Ödemkrankheit. *Der praktische Tierarzt,* 5, 440—442.

13. Wilson, R. A., Francis, D. H., 1986: Fimbriae and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with colibacillosis. *Am. J. Vet. Res.,* 47, 213—217.

Received August 20, 2003

FOLIA VETERINARIA, 47, 4: 207—209, 2003

OREGANO *(Origanum vulgare)* DIETARY SUPPLEMENTATION INCREASES THE REPRODUCTIVE PERFORMANCE OF SOWS (A Short Communication)

Kovac, B., Bilkei, G.

Bilkei Consulting, Raubbühlstrasse 4, CH-8600 Dübendorf Switzerland

ABSTRACT

In order to confirm the effect of oregano feed supplementation, the present study was performed on a large commercial herd in Eastern-Europe. We examined sows in this unit, where alternate farrowing groups were given diets containing 1,000 ppm oregano (Oregpig® Pecs, Hungary) in their lactation diet. The oregano treated groups showed a lower sow mortality rate, a lower sow culling rate during lactation, an increased farrowing rate, more liveborn piglets and less stillbirth piglets per litter compared with untreated females. The implication is that dietary oregano supplementation improves sows' reproductive performance.

Key words: dietary supplementation; oregano; reproductive performance; sow

INTRODUCTION

Sow mortality markedly influences the economics of pig breeding units (28). According to Zimmermann (22) antimicrobial feed additives reduce infectious sow mortality. According to our observations, the European restrictions of antimicrobial feed additives were followed by an increased sow mortality due to urogenital infectious diseases (6). Similar trends have been recognised in the United States (9, 16).

Antimicrobial feed additives benefit production by increasing profitability (5, 8, 11), reducing animal wastes into the environment (18) and diminishing pathogen carriage (17). As a successful alternative to prophylactic use of antibiotics against swine dysentery, phytogenic feed additives are of similar value (1). Natural and cheep feed additives, that do not bear the danger of residual substance and environmental wastes are important possibilities to improve meat production. Oregano feed supplementation positively influences daily feed intake, daily gains and feed utilization (13, 7, 12).

Our previous experiences have shown improvements in farrowing rate due to oregano feed supplements to the sow (2). Khajarern and Khajarern (15) have stated that origanum essential oils not only act as alternative antibacterial performance promoters, digestion aids and appetite enhancers in sows but also as a natural feed additive enhances growth and reproductive performance in sows.

The present study was designated to examine the effect of the strategic addition of oregano to the lactation diet under field conditions.

MATERIAL AND METHODS

The trial was performed in Alföld in Hungary from 1999 to 2002. A large indoor herd of 2,800 sows of similar genetic type, typical for this geographic area (F1 Large white x Landrace mated to Duroc boars) was selected for the study. Replacement gilts were introduced from the nucleus herd aged from 140 to 150 days. The annual replacement rate varied from 41 to 47 % between 1989 and 1998. The animals were bred at their first observed standing oestrus.

Farm management included weaning after three weeks of lactation, a group rotation system of thirty females, double artificial insemination and culling after parity 7.

The sows were kept in high investment facilities according to the requirements of the intensive breeding enterprises of the early 1990's (farrowing crates, individual gestation crates, eros center). The sows were artificially inseminated (AI) at their first post-weaning oestrus, and were moved after positive pregnancy diagnosis into gestation barns. At the 110th day of pregnancy the sows were relocated in farrowing barns where they spent the whole three weeks of lactation. Sow mortality rates of 6.7—8.1 % *per annum* had been recorded during the previous years (1989—1998) in this herd. The unit had a pre-trial (1998) annual culling and removing rate of 45.3 % (15.3 % reproductive reason, 9.5 % locomotor problems, 5.7 %

torsion abdominal organs, 4.7 % heart failure, 6.6 % culling due to old age, 3.5 % miscellaneous causes).

The sows were fed as follows:

– from mating to day 89 of gestation two kilograms, from day 90—114 of pregnancy three kilograms of a commercial gestating ration containing 12.2 MJ DE.kg^{-1}, 125 g.kg^{-1} crude protein, 6.5 g.kg^{-1} lysine, 8 g.kg^{-1} calcium and 6 g.kg^{-1} phosphorus,

– from the first day of parturition the sows were fed *ad libitum* with a lactating ration containing 13.0 MJ DE.kg^{-1}, 180 g.kg^{-1} crude protein, 10 g.kg^{-1} lysine, 8 g.kg^{-1} calcium and 6 g.kg^{-1} phosphorus,

– from weaning to mating the sows received *ad libitum* the same lactating ration, supplemented by 500 grams of potato starch/sow/day ("flushing") (4).

We examined the sows in this unit, where alternate farrowing groups were given diets containing 1000 ppm oregano feed supplementation (Oregpig® Pecs, Hungary) in the lactation diet. Oregpig® is dried leaf and flower of *Origanum vulgare*, enriched with 500 g.kg^{-1} could pressed essential oils of the leaf and flower of *Origanum vulgare*. Analysis of Oregpig®: 60 g carvacrol and 55 g thymol.kg^{-1}.

The current report involves

– fifty oregano treated farrowing groups of thirty sows each (n = 1500 sows, average parity of 2.91 (± 0.42 SD), average body condition 3.1 (± 0.12 SD) and

– untreated control animals (n = 1499, average parity 3.08 (± 0.31 SD), average body condition 3.3 (± 0.28 SD).

Body condition scores were performed according to Bilkei and Biro (3).

All farrowing barns had identical management and nutrition. Piglets were cross fostered within twenty-four hours of birth to equalise litter size from nine to eleven piglets. The average voluntary daily feed intake was 5.6 kilograms (± 0.89 SD) in primiparous (oregano group 5.6 ± 0.82 SD, control 5.6 ± 0.87 SD) and 7.3 kg (± 0.29 SD) (oregano group 7.5 ± 0.12 SD, control 7.0 ± 0.31 SD) per multiparous sow per day. The sows' weight loss and "back fat loss" during lactation has not been recorded. The litters were weaned at 21.6 ± 1.1 SD in the oregano group and at 2.9 ± 2.0 SD days of lactation in the control group.

On the day of weaning no feed was given. From day two post-weaning until breeding the sows all groups received *ad libitum* the same lactation diet, supplemented with 300 IU vitamin E per kg feed. Oestrus detection began on day three post-weaning.

The possible outcome after farrowing was that the sow died, was culled, was bred but returned to oestrus or that she farrowed again. If she farrowed another litter, the subsequent litter size was examined.

Statistical analyses were performed using the GLM procedures of SAS® (*SAS Institute, Inc.; Cary, North Caroline, USA; 19*) to derive least-squares means and standard errors. The class included in the model was parity, and the dependent variables were weaning to estrus interval (WEI) and subsequent litter size. The month of weaning, body condition at birth and lactation feed intake were found significant and included as a covariate. Full lactation length, full litter size suckled, the numbers of piglets removed and number of piglets remaining with the sow had no significant effects on WEI or subsequent litter size and therefore were not included as covariates. Sow mortality and culling rate were examined by logistic regression. The stillbirth effect was examined using a multivariate Poisson regression, controlling parity and genetic line. Farrowing rates were compared by Chi-square.

RESULTS AND DISCUSSION

The improvement of reproductive parameters illustrate the effect of adding oregano to the lactation diet. Controlling confounding factors as sow line, parity, farrowing barn, oregano supplementation to the lactation diet decreased

– sows mortality by P = 0.003 (4.21 % *vs.* 6.97 %),

– sow culling rate during lactation by P = 0.03 (oregano group 12.42 ± 1.22 % *vs.* control 15.46 ± 1.21 %),

– stillbirth rate by P = 0.05 (0.91 ± 0.01 *vs.* 0.81 ± 0.01),

– farrowing rate was increased by P = 0.01 (68.71 ± 2.42 % *vs.* 63.23 ± 2.04 %),

– more (P = 0.05) liveborn piglets per litter (oregano group 10.9 ± 1.03 *vs.* control 9.81 ± 1.12 .

The principal reasons for culling the sows were anoestrus (oregano group 14 % *vs.* control 16 % of cullings) and locomotor problems (oregano group 23 % *vs.* control 22 % of cullings). Swine urogenital disease (oregano group 11 % *vs.* control 21 %), periparturient diseases (*mastitis-metritis-agalactia*; MMA: oregano group 16 % *vs.* control 25 % cullings) heart failure (oregano group 26 % *vs.* control 10 % of cullings) and miscellaneous reasons (oregano group 8 %, control 10 % of cullings) were registered.

The majority of culling due to anoestrus took place in September and during the winter months, indicating that adverse environmental conditions (summer heat and a cold-wet winter) suppress reproductive activity. Swine urogenital disease and MMA occur often in the intensive management systems (5). In a study (14) swine urogenital disease compassed 32.4 % of sow deaths and MMA occurred in 40 % of births in a large East European breeding company.

In the present study oregano application diminished swine urogenital disease caused sow deaths by 66.6 % and the occurrence of MMA by 64 %. There exists no literature data on the possible effect of oregano on urogenital diseases and MMA of the sow. A possible therapeutic effect of oregano can only be speculative.

Phytogenic feed additives as growth promoters are controversially discussed in the literature (3). In the present trial, the effect of a phytogenic feed additive on sows reproductive performance was evaluated. It has been stated that oregano stimulates organic and micro-biotical digestion (10). Khajarern and Khaja-rern (15) have stated that carvacrol and thymol have an effect on the upper layer of mature enterocytes and accelerate the renewal rate of mature enterocytes at the

surface of the villi of the intestine. This would reduce pathogen contamination of enterocytes and improve nutrient absorption capacity.

Oregano supports the digestion and regulation of gastrointestinal metabolism (13) and exerts antibacterial properties by hindering dysbiotic processes in the digestive tract of pigs (20, 17, 21). A study has stated that 1000 ppm-dose oregano feed supplementation during the post-weaning period significantly improves weight gain and health of the pigs (12). A study (15) has stated that origanum essential oils not only act as alternative antibacterial performance promoters, digestion aids and appetite enhancers in sows but also as a natural feed additive enhancing growth and reproductive performance in sows. It has been stated (15) that supplementation of origanum essential oils in lactation feed increases ($P < 0.05$) daily feed intake and milk production.

The effect of oregano on sow fertility may only be speculative. If oregano stabilises gut microflora, decreases populations of undesirable micro-organisms, and increases the digestibility of feed, the general health of the sows may be improved and postparturient immune system activation may be positively influenced (diminishing "delayed immune response" and "reduced leukocytary activity" of the sows uterus (2). Such an effect may improve uterine involution and protect the sow from possible urogenital infections *post partum.*

A critical remark: The fact that the feed additive is of natural origin does not make it better or safer than other additives. Many antibiotics are of natural origin, originating from molds. There is no evidence that oregano would be more user-friendly than antibiotics, particularly if it exerts antimicrobial effects. Therefore, basic research is needed both to clarify the effect of oregano on the gastrointestinal, immune and urogenital system and its possible residual problems.

REFERENCES

1. Baumann, B., Bilkei, G., 2003: Effect of dietary oregano extract on the development of swine dysentery in a pure-culture challenge model (in press: *Biol. Tiermed.*).

2. Bilkei, G., 1995: Herd health strategy for improving the reproductive performance of pigs. Proc. 8th "In-between" Symposium of the International Society for Animal Hygiene. *Hung. Vet. J.,* 10, 766—768.

3. Bilkei, G,, Biro, O., 1995: Sow Parity: An economically important determinant of culling policies in intensive (large scale) pig production. *3rd Hungarian Animal Reproduction Meeting, Papers Plenary and Poster Sessions,* 115—118.

4. Bilkei, G., Bölcskei, A., 1993: Die Auswirkung der Fütterung im letzten Trächtigkeitsmonat auf die perinatalen Parameter bei verschiedener Körperkondition und Parität der Muttersauen. *Tierärztl. Umschau,* 48, 629—635.

5. Bilkei, G., Bölcskei, A., 1995: Production related culling strategy in a large pig production unit. *Pig Journal,* 35,140—149.

6. Bilkei, G., Bölcskei, A., Goos, T., 1995: Pathogene Befunde aus dem Urogenitaltrakt ausgemerzter Muttersauen aus einem Grossbestand. *Tierärztl. Prax.,* 23, 37—41.

7. Bilkei, G., Gertenbach, W., 2001: Retrospektive Untersuchung des Kombinationseffektes höher Vitamin E- und pflanzlicher Oregano-Futterzusätze auf die Entwicklung von verzögert wachsenden Mastschweinen. *Biol. Tiermed.,* 3, 83—87.

8. Cromwell, GL., Davis, G. W., Morrow, W. E., Primo, R. A., Borzeboom, D. W., Simos, M. D., Staisiewske, E. P., Ho, CH., 1996: Efficacy of the antimicrobial compound U-82, 127 as a growth promoter for growing-finishing pigs. *J. Anim. Sci.,* 74, 1284—1287.

9. Deen, J., Xue, J. L., 1999: Sow mortality in the USA: An industrywide perspective. *Proc. of the Allen D. Leman Conference,* St. Paul, Minnesota, 9—94.

10. De Koning, W., Ding Hong Biao, Wu Xian, F., Rong, Y., 1999: *Chinese Herbs in Animal Nutrition.* Nottingham University Press, pp. 7—83.

11. Duran, C. O., Walton, W., 1998: Survey of mortality in pig breeding herds. *Proc. 15th IPVS Congress, Birmingham,* 3, 235.

12. Gertenbach, W., Bilkei, G., 2001: Der Einfluss von pflanzlichen Futterzusatzstoffen in Kombination mit Linolensäure auf die immuninduzierte Wachstumsverzögerung nach dem Absetzen. *Biol. Tiermed.,* 3, 88—92.

13. Günter, K. D., Bossow. H., 1998: The effect of etheric oil from *Origanum vulgaris* (Ropadiar) in the feed ration of weaned pigs on their daily feed intake, daily gains and food utilization. *Proc. 15th IPVS Congress, Birmingham,* 223.

14. Karg, H., Bilkei, G., 2002: Causes of sow mortality in Hungarian indoor and outdoor pig production units. *Berl. Münchn. Tierärztl. Wschr.,* 115, 366—368.

15. Khajarern, J, Khajarern, S., 2002: The efficacy of origanum essential oils in sow feed. *International Pig Topics,* 17, 17.

16. Koketsu, Y., 1999: Increased sow mortality observed in the PigChamp database. *Proc. of the Allen D. Leman Conference,* St. Paul, Minnesota, p. 39

17. Kyriakis, S. C., Sarris, K., Kritas, S. K., Tsinas, A. C., Giannakopoulos, C., 1996: Effect of salinomycin in the control of *Clostridium perfringens* type C infections in suckling pigs. *Vet. Rec.,* 138, 281—283.

18. Roth, F. X., Kirchgessner, M., 1993: Influence of avilamycin and tylosin on retention and excretion of nitrogen in finishing pigs. *J. Anim. Physiol. Anim. Nutr.,* 69, 245—250.

19. SAS/STAT® User's Guide, Version 6. Cary, North Carolina: SAS Institute Inc. 1988.

20. Sivropoulou, A., Papanikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T., Arsenakis, M., 1996: Antimicrobial and cytotoxic activities of origanum essential oils. *J. Agr. Food Chem.,* 44, 1202—1205.

21. Tsinas, A. C., Kyriakis, S. C., Bourtzi-Chatzopoulou, E., Arsenakis, M., Sarris, M., Papasteriades, A., Lekkas, S., 1998: Control of porcine proliferative enteropathy by feed application of Oreganum essential oils. *Proc. 15th IPVS Congress, Birmingham,* 220.

22. Zimmermann, D. R., 1986: Role of subtherapeutic levels of antimicrobials in pig production. *J. Anim. Sci.,* 62, 6—17.

Received May 21, 2003

FOLIA VETERINARIA, 47, 4: 210—211, 2003

ILLNESS IN PERIPARTURIENT SOWS, CAUSED BY *Clostridium difficile*
(A Short Communication)

Mauch, C. P., Bilkei, G.

**Artgerechte Tierhaltung, Meiengarten 1, 8645 Jona
Switzerland**

ABSTRACT

In a German outdoor pig production unit a sudden increase of postparturient sow mortality was diagnosed. In order to prevent the disease, prophylactic *post partum* enrofloxacine injections were carried out. Despite this therapy, diarrhoea, respiratory distress and mortality of sows were registered. Gross pathology of dead sows, microscopic examination, culture and enzyme immunoassay revealed a *Clostridium difficile* infection.

Key words: *Clostridium difficile*; morbidity; mortality; postpartum

Clostridium (C.) difficile is a Gram-positive to Gram-variable, spore-forming anaerobe which is commonly found in the intestinal tract of various mammals, birds and reptiles (6). In typical cases, *C. difficile* infection may occur due to stress, lack of hygiene and as a result of exposure to antimicrobials (2). *C. difficile*-caused postparturient sow losses have never been reported in the literature.

In Germany a large outdoor production unit with unfavourable environmental conditions, a sudden increase of therapy resistant mastitis-metritis-agalactia (MMA) and postparturient sow mortality was reported in February 2003. Postparturient (p.p.) fever (>40.0 °C) was in this unit the diagnostic criteria (1) for MMA. The sows (n = 128) having suffered fever (>40.0 °C) received as p. p. MMA prophylaxis, within six hours p. p. and after twenty-four hours a second time, Enrofloxacine (Baytril®, Bayer, Munich, Germany, 3 mg.kg^{-1} body weight) injections. Despite rapidly declining fever (<39.5 °C) days 2 and 3 p.p., diarrhoea and sudden respiratory distress was reported in 31 of the 128 animals.

Twenty one sows died. Seven dead sows were submitted for necropsy.

The gross pathology of the small intestine revealed congestion of the submucosa and mucosa. Large intestinal contents were yellov to dark yellov. All sows had highly oedematous spiral colons. Microscopic examination of the small intestine revealed scattered suppurative foci in the colonic *lamina propria*. Neutrophilic infiltrates were found in the mesocolon. The colonic mucosa showed segmental erosions and "vulcano-like" lesions (neutrophil and fibrin exsudation into the colonic lumen). Gram stains of colonic smears revealed large numbers of Gram-positive rods. Anaerobic cultures of the colon yielded high numbers of *C. difficile*. Enzyme immunoassay revealed toxins A and B (toxin testing of faecal samples by neutralisation in Chinese hamster ovary cells (2)and by commercial enzyme immunoassay. The above listed cumulative findings confirmed *C. difficile*-caused sow mortality.

Sow mortality may represent high losses to the outdoor production herd (4). Sows appear to be at risk of death during the early p. p. period (5). According to a Hungarian study, in indoor production units 19.4 % and in outdoor ones 40.1 % of sow mortality occurred during the peripartal period (4). In a Canadian study on sow mortality, 42 % of all deaths occurred during the peripartum period (4). Similarly, Schultz *et al.* (5) have stated that 39 % of sow mortality occurred in a seven-day time period from two days before to five days after farrowing. Factors that place sows in a weakened condition during the early p. p. period include retention of placenta or retained pigs, physical injury in a sub-optimal outdoor environment, infections, impaired immunological response and inadequate early lactational feed intake (1). In a study (5) forty percent of dead sows were cultured positive for *Clostridium noviy.*

The diagnosis of *C. noviy* sudden death is difficult, mainly because this organism is a common and early postmortem invader, especially in warm weather. Although *C. difficile* has been reported as a cause of neonatal piglet diarrhoea, it is difficult to assess the importance of this pathogen to the swine industry (1). *C. difficile* is considered to be an opportunist (1). One method of controlling an opportunist is to understand the risk factors associated with disease and eliminate those factors. The possible effect of antibiotics on the development of *C. difficile* associated sow mortality may have had similar pathogenic mechanism as *C. difficile* caused antibiotic-associated diarrhoea (6) in humans.

While in the present case the 128 MMA affected sows had similar parity distribution as the whole unit, the sows that died were significantly younger ($p < 0.05$). This data indicate that the older sows probably may have had a better immune status against *C. difficile* infections. The fact that during the study period (January-February 2003) no excessive preweaning piglet losses ($14.5 \pm 4.2\%$) were registered, supports the theory that not *C. difficile* alone but an antibiotic application to the p. p. sows could have been one of the triggering factors for *C. difficile*-caused sow mortality. In the present case, *C. difficile*-caused sow mortality disappeared after p. p. enrofloxacine injections were stopped. It cannot be ruled out that a sporadic outbreak of *C. difficile* disease occurred and was self-limiting.

Conclusions: *Clostridium infections of postparturient sows may be associated to environmental stress and / or to application of antibiotics.*

REFERENCES

1. Bilkei, G., 1995: Herd health strategy for improving the reproductive performance of pigs. *Hung. Vet. J.,* 10, 766—768.

2. Brazier, J. S., Borriello, S. P., 2000: Microbiology, epidemiology and diagnosis of *Clostridium difficile* infection. In **Aktories, K. Wilkens** (ed.): *Clostridium difficile.* Springer, Berlin TC, pp. 11—18.

3. Chagnon, M., D'Allaire, S., Drolet, R., 1991: A prospective study of sow mortality in breeding herds. *Can. J. Vet. Res.,* 55, 180—184.

4. Karg, H., Bilkei, G., 2002: Causes of sow mortality in Hungarian indoor and outdoor pig production units. *Berl. Münch. Tierärztl. Wschr.,* 115, 366—368.

5. Schultz, D., Dau, D., Cast, W., 2001: A sow mortality study – the real reasons sow die identifying causes and implementing action. *Proc. Am. Ass. Swine Vet.* (Des Moines), 387—395.

6. Songer, J. G., Post, K. W., Larson, D. J., Jost, B. H., Glock, R. D. 2000: Infection of neonatal swine with *Clostridium difficile. Swine Health and Production,* 4, 185—189.

Received July 17, 2003

FOLIA VETERINARIA, 47, 3: 212—214, 2003

THE GROWTH OF *Geotrichum candidum* AT DIFFERENT TEMPERATURES AND SODIUM CHLORIDE CONCENTRATIONS

Pažáková, J., Pipová, M., Nagy, J., Turek, P.

**University of Veterinary Medicine, Komenského 73, 041 81 Košice
The Slovak Republic**

pazakova@uvm.sk

ABSTRACT

The dairy industry tries to achieve product uniformity by adding starter cultures. The so-called cheese-starter cultures usually comprise selected *Penicillium* spp. and *Geotrichum candidum*. In general, yeasts and *Geotrichum candidum* are believed to play an important role in the development of the aromatic profile of cheeses. The effect of ambient temperature on the growth of *Geotrichum candidum* was tested on the surface of Sabouraud's agar. The plates were incubated at different temperatures for 24, 48, 72, and 96 hours. It was found that *Geotrichum candidum* grows at temperatures between 5 °C and 38 °C. The maximum growth rate was observed between 28 °C and 30 °C. The inhibitory effect was already observed by a 0.5 % concentration of sodium chloride. The growth of *Geotrichum candidum* was completely stopped by a 6.5 %-salt-concentration in the culture medium.

Key words: *Geotrichum candidum*; milk; milk products; temperature; sodium chloride

INTRODUCTION

Geotrichum candidum (in dairy technology sometimes called *Oospora lactis* or *Oidium lactis*) is an ubiquitous mould of the family Moniliaceae. In human medicine, *Geotrichum candidum* is considered to be a conditionally pathogenic mould which can cause disease under specific conditions, mainly in patients whose immunity has been considerably lowered by some chronic diseases (11).

The production of standard cheeses requires control of the diversity of microflora, especially in the cheese-ripening phase. Industrial cheese-making first removes all non-desirable micro-organisms by pasteurization, and then tries to achieve product uniformity by adding starter cultures. The species of micro-organisms used in the starter cultures results in enormous variations in the final organoleptic properties of the cheeses. The majority of flavour and odour compounds involved in the final sensory quality results from microbial proteolysis and lipolysis (8).

In general, yeasts and *Geotrichum candidum* are believed to play an important role in the development of the aromatic profile of cheeses (5). The so-called cheese-starter cultures in the dairy industry, besides three *Penicillium* species (*P. roquefortii*, *P. camembertii*, and *P. nalgiovense*), comprise *Geotrichum candidum* (10, 14). *Geotrichum candidum* influences the cheese-texture making the cheese more meltable, fatty, and creamy, as well as reducing its bitterness.

It is known that *Geotrichum candidum* excretes D-3-phenyllactic acid which inhibits the growth of *Listeria monocytogenes*. It has been found capable of inhibiting a range of undesirable Gram-positive bacteria in man and foofstuffs, such as *Staphylococcus aureus* and *Enterococcus faecalis*, as well as such Gram-negative bacteria as *Providencia stuartii* and *Klebsiella oxytoca* (2). Dual starter cultures involving *Geotrichum candidum* also inhibit the production of mycotoxins (10).

On the other hand, this mould is a common contaminant of milk and milk products and its presence can cause a lot of undesirable organoleptic changes in milk products, especially in some kinds of mould-ripened cheese (1, 12).

MATERIALS AND METHODS

The influence of ambient temperature on the growth of *Geotrichum candidum* was tested on the surface of Sabouraud's dextrose agar. Each plate was inoculated with the help of an inoculating needle in three different places. The plates were

then incubated at temperatures listed in Table 1. The colony sizes were measured after 24, 48, 72, and 96 hours of incubation.

The effect of sodium chloride on the growth of *Geotrichum candidum* was also determined using agar according to Sabouraud. Different amounts of sodium chloride were added to each 100 ml of the culture medium in order to achieve the final concentration of salt as required (e.g. the addition of one gram of sodium chloride to 100 ml of the agar resulted in a 1 %-concentration). *Geotrichum candidum* was inoculated on the surface of agar containing different amounts of salt with the help of an inoculating needle. Plates were then incubated at a temperature of 24 °C for 24, 48, and 72 hours, when the size of colonies was measured.

RESULTS

As seen from the results in Table 1, no growth of *Geotrichum candidum* was observed at 4 °C. The strain used in the experiment started to grow at a temparature of 5 °C. Significant growth acceleration was noticed at 19 °C and a maximum growth rate was observed between 28 °C and 30 °C. At higher temperatures the growth of *Geotrichum candidum* continuously decreased and it stopped completely at a temperature of 39 °C. The relationship between the size of the colonies and both the time and the temperature of incubation is shown in Table 1.

The relationship between the size of the colonies, the time of incubation, and the concentration of sodium chloride in the culture medium is given in Table 2. The inhibitory effect was already observed by a 0.5 % concentration of sodium chloride in the agar. The growth of *Geotrichum candidum* was completely stopped by a 6.5 %-salt-concentration in the culture medium.

DISCUSSION

The increasing use of bacteria and yeast strains with flavour-enhancing enzyme activity, especially proteolytic, in cheese production allows the reproduction of traditional flavours or meets consumer demand for new flavours.

Storage at low temperatures (–9 and –18 °C) does not cause 100 %-mortality of the *Geotrichum candidum* spores. The proportion of dead cells increases with the decrease in temperature. Prolonged exposure to low temperature causes an increase in the mortality rate of the arthrospores. However, some of them can survive a 24-hour or even 7-day incubation at –18 °C (1). On the other hand, any milk pasteurization results in the devitalization of *Geotrichum candidum* (16).

The ability of *Geotrichum candidum* to grow at different temperatures has already been published in 1963 (9). In this study, the growth of *Geotrichum candidum* at temperatures of 37 °C, 30 °C, 25 °C, and 20 °C within 1 to 3 days was noticed, with an optimum growth rate at 30 °C, but also in some cases at 37 °C or 25 °C. The average diameter of *Geotrichum candidum* colonies ranged from 24 to 67 mm. *Geotrichum candidum* is reported to grow much quicker than other *Geotrichum* species.

Furthermore, different data have been reported for two types of *Geotrichum candidum* strains (6). For the yeast form of *Geotrichum candidum*, the optimum lies between 22 °C and 25 °C. The growth of those strains is considerably reduced at a temperature of 30 °C. On the other hand, the mould strains of *Geotrichum candidum* show the best growth between 25 °C and 30 °C.

No or minimum (up to 25 %) growth of *Geotrichum candidum* has been reported at 37 °C and a maximum growth at 24 °C (4). The range of a *Geotrichum candidum* growth lies between 5 °C and 35 °C (13) or between 4 °C and 38 °C (7) with an optimum at 25 °C (13) or at 28 °C (7). An 80 %-growth of *Geotrichum candidum* strains at 28 °C, an 87—100 %-growth at 37 °C, and a 13 %-growth at 42 °C have also been observed (15).

As to the effect of sodium chloride on the growth of *Geotrichum candidum*, little data can be found in current literature. In general, the majority of moulds are

Table 1. The effect of ambient temperature on the growth of *Geotrichum candidum*

Temperature (°C)	The average sizes of colonies (mm) after 24 hours	48 hours	72 hours	96 hours
4	0.0	0.0	0.0	0.0
5	0.5	1.0	2.0	3.0
6	0.5	1.0	2.0	3.0
8	2.0	5.0	9.0	12.0
13	2.0	5.0	10.0	13.0
16	4.0	8.0	13.0	18.0
19	7.0	16.0	23.0	29.0
21	8.0	18.0	24.0	30.0
24	9.0	20.0	25.0	38.0
28	10.0	20.0	27.0	40.0
30	10.0	21.0	30.0	40.0
32	6.0	14.0	21.0	28.0
35	1.0	2.0	3.0	4.0
38	0.5	1.0	2.0	3.0
39	0.0	0.0	0.0	0.0

Table 2. The effect of sodium chloride on the growth of *Geotrichum candidum*

% of sodium chloride	The average sizes of colonies (mm) after 24 h	48 h	72 h
0.0	9.0	17.0	25.0
0.5	8.0	16.0	23.0
1.0	6.0	12.0	20.0
1.5	5.0	10.0	18.0
2.0	4.0	9.0	15.0
2.5	4.0	8.0	13.0
3.0	3.0	6.0	10.0
3.5	3.0	5.0	9.0
4.0	2.0	4.0	6.0
4.5	2.0	3.0	5.0
5.0	1.0	2.0	3.0
5.5	0.5	1.0	2.0
6.0	0.0	0.5	1.0
6.5	0.0	0.0	0.0
7.0	0.0	0.0	0.0
7.5	0.0	0.0	0.0

reported to be more sensitive than the majority of yeasts. *Geotrichum candidum* is mentioned as one of the moulds most sensitive to the presence of sodium chloride (3).

The presence of 1 % sodium chloride already results in growth inhibition. However, the growth of *Geotrichum candidum* is not stopped by a salt content between 5 and 6 % (13).

CONCLUSION

It should be taken into account, that *Geotrichum candidum* is not only a desirable mould widely used in the cheese-making industry, but it is also a common milk contaminant. Due to its enzymatic activity it can be responsible for many undesirable organoleptic changes in milk products developing in their processing or storage before consumption. As follows from results, the range of temperatures used in dairy industry in the production and storage of milk and milk products, as well as the concentration of sodium chloride used in cheese-making industry do not cause the devitalization of *Geotrichum candidum. Therefore, this mould can grow and develop on the surface of milk products and result in their condemnation.*

REFERENCES

1. Bielasiewicz, D., 1996: Effect of low temperatures on the survival of cells and activity of some oxidoreductases of *Geotrichum candidum* Link (In Polish). *Chlodnictwo,* 31, 28—31.

2. Dieuleveux, V., Lemarinier, S., Gueguen., M., 1998: Antimicrobial spectrum and target size of D-3-phenyllactic acid. *Int. J. Food Microbiol.,* 40, 177—183.

3. Doležálek, J., 1962: *Microbiology of the Dairy and Fat Manufactures* (In Czech) (1st edn.). Publ. House SNTL Praha, 1962.

4. Frágner, P., 1984: *Small Encyclopedia of Medicine* (In Czech) (1st edn.). Publ. House Avicenum Praha, 183 pp.

5. Gobin, F., 1999: Technologie fromagere. Reusssir le croutage des fromages. *Chevre,* 170, 37—39.

6. Gueguen, M., Jacquet, J., Lemarinier, S., 1983: Sur la morphologie de *Geotrichum candidum* Link et sa variabilite. *Microbiologie-Aliments-Nutrition,* 1: 49—57.

7. Jesenská Z., 1987: *Microscopic Fungi in Food- and Feedstuffs* (In Slovak) (1st edn.). Publ. House Alfa Bratislava, 319 pp.

8. Molimard, P., Lesschaeve, I., Issanchou, S., Brousse, M., Spinnler, H. E., 1997: Effect of the association of surface flora on the sensory properties of mould-ripened cheese. *Lait,* 77, 181—187.

9. Morenz, J., 1963: *Geotrichum candidum* Link, Taxonomie, Diagnose und medizinische Bedeutung. *Mykol. Schr.-R.,* 1, 7—79.

10. Nielsen, M. S., Frisvad, J. C., Nielsen, P. V., 1998: Colony interaction and secondary metabolite production of cheese-related fungi in dual culture. *J. Food Protect.,* 61, 1023—1029.

11. Schönborn, Ch., Hourieh-Zaza, E. M., Haustein, U. F., Rytter, M., 1982: Serologischer Nachweiss einer Sensibilisierung durch *Geotrichum candidum. Mycosen,* 25, 662—673.

12. Shimada, T., Ichinoe, M., 1998: Fungal species from imported and domestic mold-ripened cheese. *J. Food Hyg. Soc. Japan,* 39, 350—356.

13. Siegbert, Ph., 1985: Spezialkulturen – Bedeutung und Einsatz in der Käserei. *Deutsche Molkerei-Zeitung,* 106, 1706—1710.

14. Skovgaard-Nielsen, M., 1999: Skimmelstarters haemning of uonsket skimmelvaekst. *Maelkeritidende,* 112, 10—11.

15. Sláviková, E., Kováčová, R., Kocková-Kratochvílová, A., Hašková, B., 1998: *The occurrence of yeasts and yeast-like organisms in camembert cheese* (In Slovak). Bull. Výsk. úst. potrav., 27, 355—364.

16. Vadillo, S., Paya, M. J., Cutuli, M. T., Suarez, G., 1987: Mycoflora of milk after several types of pasteurization. *Lait, 67, 265—273.*

Received July 8, 2003

FOLIA VETERINARIA, 47, 4: 215—217, 2003

THE EFFECT OF NATURAL ANTIOXIDANTS ON OXIDATIVE PROCESSES IN PORK MEAT

**Marcinčák, S., Bystrický, P., Turek, P.
Popelka, P., Máté, D., Sokol, J., Nagy, J., Ďurčák, P.**

**University of Veterinary Medicine, Komenského 73, 041 81 Košice
The Slovak Republic**

marcincak@lycos.com

ABSTRACT

The influence of natural antioxidants, rosemary extract (in 96 % ethanol), in combination with ascorbic acid and lactic acid, respectively, was studied. Their antioxidant effect on unsalted pork chops stored for 24, 48, 72, 96, and 144 h at 4 °C was estimated. Both combinations of antioxidants had an antioxidative effect expressed in a lower peroxide value (PV) and slower increase of thiobarbituric value (TBA) in comparison with the control sample without antioxidants ($P < 0.05$). The combination of rosemary with ascorbic acid had the highest oxidation suppressive effect on cut meat.

Key words: antioxidant; ascorbic acid; pork meat; rosemary extract

INTRODUCTION

The processing of meat products (grinding, mixing, cutting, and thermal treatment) destroys the muscle membrane system with subsequent oxidation of intracellular lipids, primarily membrane phospholipids. Pre–processing of meat causes the initiation of oxidative processes and production of free radicals and hydroxyperoxides from fatty acids (3). The process of oxidation continues during thermal treatment and storage when the second phase and third phase of lipid autooxidation, propagation and termination, take place, during which hydroperoxydes are formed from lipids and subsequently de-composed giving rise to a broad set of compounds. These negatively influence the sensory properties of food and thus their shelf life (2).

Lipid oxidation is considered as the main cause of some "civilization diseases", such as heart and vessels diseases (arteriosclerosis, heart failure), brain damaging diseases (Parkinson, Alzheimer diseases) and cancer.

Some possibilities of fat protection against oxidation were found. The elimination of oxygen and pro-oxidants from meat under processing, using packaging in modified atmosphere or vacuum or simply in materials, which protect food against atmospheric oxygen. Lowering the temperature also slows down lipid oxidation. However, the most effective way of oxidative process inhibition is the addition of antioxidants.

Synthetic antioxidants – butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallates, are used routinely in practice (6, 7). However, their use is limited because of possible adverse effects and because of the negative attitude of consumers. Therefore natural antioxidants are used in preference. Some vegetables and spices are known antioxidants and are the subject of intensive research. In most spices there are flavonic and phenolic compounds that are responsible for an antioxidative effect (8). The principle is interruption of the auto-oxidation chain. For the antioxidative effect of rosemary, the flavonic components, carnosol acid, carnosol and rosemary acid are responsible (8).

The goal of our experiment was to estimate the anti-oxidative effect of rosemary extract in combination with lactic acid and ascorbic acid in cut meat for meat products. Along with that, changes in the quality of chilled pork fat were estimated.

MATERIAL AND METHODS

Rosemary extract was prepared by extraction from dry leaves of rosemary (*Rosmarinus officinalis* L.) in 96 % ethanol according to Bendini *et al. (1)*. Extraction was conducted at boiling temperature, in a Soxhlet apparatus, for four hours.

A pork thigh was frozen at –18 °C 24 hours after processing and stored one month. Frozen meat was cut and divided into three groups. Group 1 (control, C) was processed without an antioxidant addition. To group 2 (A) 2 % rosemary extract

and ascorbic acid were added in concentrations 1 ml.kg^{-1} and 100 mg.kg^{-1}, respectively. In group 3 (M), 10 % lactic acid concentration of 30 ml.kg^{-1} was used instead of the ascorbic acid, again in combination with the rosemary extract. The meat from all groups was cut and mixed for five minutes, put into oxygen permeable plastic bags and chill stored at 4 °C.

The first analysis was performed immediately after mixing the meat. Subsequent examinations followed after 24, 72, 96, and 144 hours of storage. Then decomposition processes of fat pork in samples were examined in terms of their peroxide value (PV), thiobarbituric value (TBA) and acid value of fats (AV). PV and AV examinations were carried out according to Veterinary Laboratory Methods (9), and TBA was analysed according to Grau *et al.* (4) with spectrophotometric detection at 532 nm (Helios γ v 4.6, Thermo spectronic, Cambridge, UK).

Statistical analysis was based on six samples per group, mean values compared by Student's *t*-test and $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The results of fat analyses carried out immediately after mixing are summarised in Table 1. Group C samples

Table 1. The Oxidative status of pork fat after cutting

Group	Parameters of examination		
	PV (mmol.kg^{-1})	TBA (μg.g^{-1})	AV (mg KOH.g^{-1})
C	47.22	0.085	9.10
A	15.00	0.049	7.42
M	25.30	0.053	5.89

C — control,
M — 2 % rosemary extract + 30 ml.kg^{-1} of 10 % lactic acid,
A — 2 % rosemary extract + 100 mg.kg^{-1} of ascorbic acid

groups M and C. The combination of rosemary with ascorbic acid had the highest oxidation suppressive effect in cut meat ($P < 0.05$).

Examination of PV in the chill stored meat samples showed that the best results were obtained from samples of group A. Its PV was stable during the whole storage period as shown in the Fig. 1. The worst results were found in group C. At the beginning of the experiment, PV was increased but twenty-four hours after cutting the meat the PV level decreased rapidly with a subsequent slow rise. Both combinations of rosemary extract, with ascorbic acid and with lactic acid, respectively, had a protective effect against the primary oxidative damage of the fat. Similar results have been reported also by Korimová *et al.* (2000).

The storage of cut meat at 4 °C had influence on its TBA value. In all three groups a slow increase in TBA value vas observed (Fig. 2). After 144 hours of chill storage, the highest TBA value, 0.085 μg.g^{-1}, was estimated

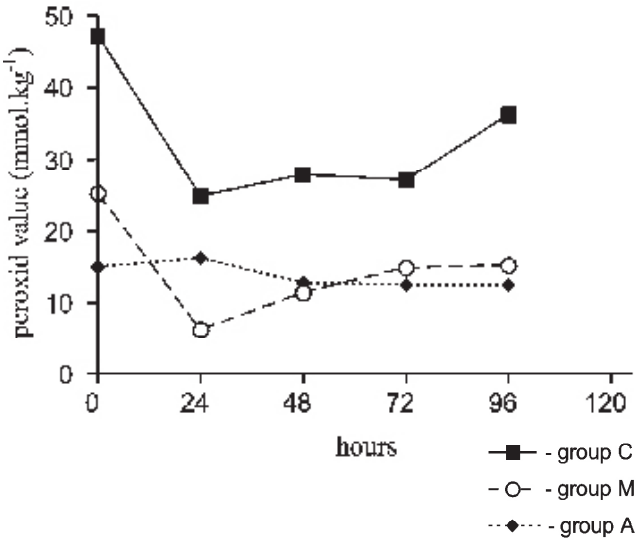


Fig. 1. Peroxide value changes during storage at 4 °C

C — control
M — 2 % rosemary extract + 30 ml.kg^{-1} of 10 % lactic acid
A — 2 % rosemary extract + 100 mg.kg^{-1} of ascorbic acid

provided the worst results in all three observed parameters. Peroxide values estimated in groups A and M, containing rosemary extract, were lower in comparison with the group C ($P < 0.05$). For the thiobarbituric value, the best result was observed in group A, followed by

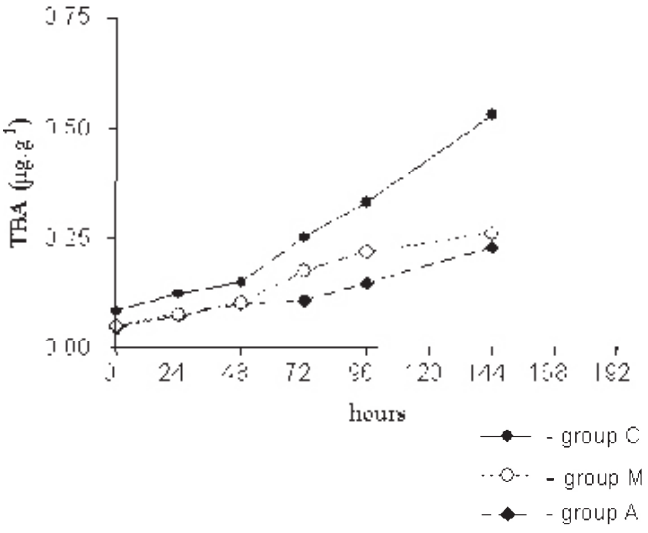


Fig. 2. TBA value changes during storage at 4 °C

C — control,
M — 2 % rosemary extract + 30 ml.kg^{-1} of 10 % lactic acid
A — 2 % rosemary extract + 100 mg.kg^{-1} of ascorbic acid

in group C. In groups A and M, TBA values 0.228 μg.g^{-1} and 0.261 μg.g^{-1} were found, respectively. These values are significantly lower in comparison with the results of the control group ($P < 0.05$). The combination of rosemary extract with both ascorbic and lactic acids showed a significant antioxidative effect. Comparable results were estimated also in preserved pork (5), poultry meat (6) and turkey meat (10).

CONCLUSION

Addition of rosemary extract in combination with ascorbic acid suppresses oxidation in pork fat. Use of antioxidants in combination is useful for meat cutting and processing because it increases the stability of its fat part.

The antioxidative effect of rosemary extract in combination with lactic acid can be used in raw fermented meat products when lactic acid is a final product of fermentation.

ACNOWLEDGEMENT:

This study was supported by a grant VEGA SR No. 1/8237/01.

REFERENCES

1. Bendini, A., Toschi, G. T., Lercker, G., 2002: Antioxidant activity of oregano (*Origanum vulgare* L.) leaves. *It. J. Food Sci.,* 14, 17—24.

2. Bystrický, P., Dičáková, Z., 1998: Animal lipids in foods (In Slovak). *Slovenský veterinársky časopis — Suplementum,* 1, 24, 1—46.

3. Eriksson, C. E., Na, A., 1995: Antioxidant agents in raw materials and processed foods. *Biochem. Soc. Symp.,* 61, 221—234.

4. Grau, A., Guardiola, F., Boatella, J., Barroeta, A., Codony, R., 2000: Measurment of 2-thiobarbituric acid values in dark chicken meat through derivate spectrophotometry: Influence of various parameters. *J. Agric. Food Chem.,* 48, 1155—1159.

5. Guntensperger, B., Hammerli-Meier, D. E., Escher, F. E., 1998: Rosemary effect and precooking effects on lipid oxidation in heat sterilized meat. *J. Food Sci.,* 63, 955—957.

6. Karpinska, M., Borowski, J., Danowska, M., 2000: Antioxidative activity of rosemary extract in lipid fraction of minced meat balls during storage in freezer. *Nahrung,* 44, 38—41.

7. Korimová, L., Máté, D., Turek, P., 2000: Influence of natural antioxidants on heat-untreated meat products quality (In Slovak). *Czech Journal of Food Science,* 18, 124—128.

8. Takácsová, M., Kristiánová, K., Nguyen, D. V., Dang, M. N., 1999: Influence of extracts from some herbs and spices on stability of rapeseed oil (In Slovak). *Bulletin of Food Research,* 38, 17—24.

9. Veterinary Laboratory Methods, 1990: *Food Chemistry* (In Czech), Štátna veterinárna správa SR, Bratislava, 130—135.

10. Yu, L., Scanlin, L., Wilson, J., Schmidt, G., 2002: Rosemary extracts as inhibitors of lipid oxidation and colour change in cooked turkey products during refrigerated storage. *J. Food Sci.,* 67, 582—585.

Received May 28, 2003

FOLIA VETERINARIA, 47, 4: 218—220, 2003

MICROBIAL ANALYSIS OF RAW MATERIALS AND HEAT-PROCESSED MEAT PRODUCTS

Laciaková, A., Pipová, M., Pástorová, B., Laciak, V.

**The University of Veterinary Medicine, Komenského 73, 041 81 Košice
The Slovak Republic**

laciakova@uvm.sk

ABSTRACT

This study focuses on the microbial analysis of selected soft, heat-processed meat products and the raw materials used for their production. Special attention has been paid to the isolation and identification of microscopic filamentous fungi. Five samples (from the surface of beef, ground beef, ground pork, salami emulsion, and final product) were taken each month within a period of one year. The microbial investigation was aimed at the determination of total plate counts, the counts of coliforms, the counts of *Staphylococcus aureus*, the presence of *Salmonella* spp. and the counts of moulds. The highest level of microbial contamination (total plate count, counts of coliforms and *Staphylococcus aureus*) was observed in both kinds of ground meat during summer months. The presence of *Salmonella* spp., was not determined in any sample. The highest counts of microscopic filamentous fungi were found in samples of salami emulsion in summer months. Toxicogenic genera (*Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp.) have also been identified.

Key words: meat products; microbial contamination; toxicogenic moulds

INTRODUCTION

Raw materials, as well as final meat products are exposed to a high risk of microbial contamination at the time of their production, processing, storage and distribution. The chemical compostition of food, properties of the outside environment and specific growth requirements determine the type of micro-organisms and the course of physico-chemical reactions in the contaminated food. Food stuffs, in general, represent an ideal medium for the persistence and multiplication of the toxicogenic filamentous fungi which possess the ability to produce mycotoxins under suitable conditions.

More than 64,000 moulds, yeasts and yeast-like organisms have been found in the environment. Among them, 114 mould and 12 yeast species are of use in the food industry. Sixty five species of the 114 reported are able to synthesize more than 150 kinds of mycotoxins. As to meat and meat products, 78 mould species have already been isolated, 50 of them being potentially toxicogenic (8). Foods contaminated with toxicogenic microscopic filamentous fungi are considered to be a reservoir of the so-called "hidden mycotoxins" (8). Therefore, an increased interest in both the quantitative and qualitative microbial examination of the meat products has been noticed worldwide (5, 6, 8).

Currently, little is known about the contamination of food with toxicogenic fungi. Therefore, the aim of this study was to perform a microbial analysis of the selected soft heat-processed meat products and raw materials used for their production. All samples were inspected for the presence and counts of different mould genera.

MATERIAL AND METHODS

In the course of one year, five samples were taken each month from the surface of beef, ground beef, ground pork, salami emulsion in a beef/pork ratio 20/80 % and soft heat-processed meat products. In each of the sixty samples, the following parameters were determined: total plate count, the count of coliforms, the presence of *Salmonella* spp. and the count and genera of microscopic filamentous fungi.

Ten grams of the sample were diluted with 90 ml of physiological saline and homogenized using the propeller homogenizer (MEZ Náchod, The Czech Republic) at 10,000 rpm for 2.5 min-

utes. Decimal dilutions of the sample were further prepared in accordance with government regulation STN ISO 560102 (12). Specific microbiological investigations followed the procedures set by the following government standards: total plate count — STN ISO 4833 (13), the count of coliforms — STN ISO 4832 (14), the count of *Staphylococcus aureus* — STN ISO 6888 (15), the presence of *Salmonella* spp. — STN ISO (16), the count of moulds — STN ISO 7954 (17).

RESULTS AND DISCUSSION

The results of this study are shown in Tables 2 and 3.

Table 2. Species of microscopic filamentous fungi isolated

Period	The species of mould
Spring months	*Penicillium* spp., *Mucor* spp.
Summer months	*Penicillium* spp., *Aspergillus flavus*, *Cladosporium* spp., *Mucor* spp., *Absidium* spp., *Fusarium moniliforme*
Autumn months	*Penicillium* spp., *Mucor* spp., *Rhizopus* spp., *Scopulariopsis* spp.
Winter months	*Penicillium* spp., *Rhizopus* spp., *Mucor* spp.

Table 3. The average counts of micro-organisms in 1 gram of samples

Period	Samples	TPC	Coliforms	*Salmonella* spp.	*Staph. aureus*	Moulds
Spring months	1	$3.8.10^4$	0	0	0	0
	2	$2.2.10^7$	$2.2.10^4$	0	0	0
	3	$4.2.10^5$	$6.2.10^5$	0	0	0
	4	$6.4.10^5$	$6.3.10^2$	0	0	0
	5	$3.8.10^5$	$1.9.10^2$	0	0	$7.5.10^1$
Summer months	1	$4.2.10^5$	0	0	0	$1.5.10^1$
	2	$1.9.10^8$	$1.0.10^5$	0	$2.8.10^4$	$4.5.10^1$
	3	$9.8.10^7$	$8.2.10^6$	0	$2.2.10^3$	$5.0.10^1$
	4	$9.2.10^6$	$6.7.10^4$	0	0	$4.0.10^2$
	5	$4.3.10^5$	$3.2.10^2$	0	0	$1.5.10^2$
Autumn months	1	$2.5.10^3$	0	0	0	0
	2	$3.1.10^6$	$8.5.10^4$	0	$1.4.10^2$	$2.5.10^1$
	3	$1.3.10^5$	$2.1.10^4$	0	$2.2.10^3$	$4.3.10^1$
	4	$2.3.10^5$	$2.5.10^2$	0	0	$2.2.10^1$
	5	$2.9.10^5$	0	0	0	0
Winter months	1	$1.8.10^2$	0	0	0	$6.0.10^1$
	2	$1.9.10^6$	$1.8.10^4$	0	$5.0.10^3$	$8.5.10^1$
	3	$2.8.10^5$	$3.2.10^3$	0	$6.5.10^3$	$2.5.10^1$
	4	$5.4.10^5$	$9.2.10^3$	0	0	$3.5.10^1$
	5	$1.9.10^4$	0	0	0	$1.2.10^1$

Legends: 1 — the surface of beef; 2 — ground beef
3 — ground pork; 4 — salami emulsion
5 — soft heat processed meat product

Meat surface is usually heavily contaminated with a wide range of micro-organisms. Due to its beneficial chemical composition (the content of water, proteins, peptides, amino acids, nucleotides, sugars, minerals and vitamins), meat is a suitable medium for the development of all micro-organisms (11). Besides various gram-negative (*Escherichia* spp., *Enterobacter* spp., *Yersinia* spp., and *Pseudomonas* spp.) and G-positive bacteria (*Bacillus* spp., *Micrococcus* spp. and *Lactobacillus* spp.), psychrotrophic moulds (*Aspergillus* spp., *Cladosporium* spp., *Geotrichum* spp., *Mucor* spp. and *Rhizopus* spp.) are frequently isolated from the meat surface (9). General characteristics and conditions for both the development of moulds and the production of

As seen from both tables the highest level of microbial contamination was observed in the summer months, when the total plate counts ranged from 10^5 to 10^8 per gram of the sample. These numbers exceeded the limits set by the Slovak *Codex Alimentarius* (10) for soft, heat-processed meat prodcuts. As to coliforms, their counts were also significantly higher during the summer. On the other hand, the presence (Tab. 3) of *Staphylococcus aureus* was determined in the summer, autumn, and winter months in samples of ground beef and pork. The maximum values set by the Slovak *Codex Alimentarius* were exceeded in one sample. The incidence of microscopic filamentous fungi showed at a maximum during the summer months. The presence of *Salmonella* spp. was not determined in any of samples inspected.

Table 1. General characteristics of mould development and the production of mycotoxins in meat and meat product (Ostrý, 2001)

Factors	Growth of moulds	Production of mycotoxin
Temperature	from –12 to 55 °C	from +4 to +44 °C
pH-value	from 1.7 to 10	from 2.5, optimum between 5 and 7
Available water	min. of 0.62	min. of 0.8—0.85
Redox potential	aerobic conditions	aerobic conditions
Addition of salt	up to 20 % NaCl	up to 14 % NaCl
Influence of spices (eugenol, anethol, thymol)	inhibition	inhibion

mycotoxins in the meat and meat products are reported in the following Table 1 (7).

It is evident, that both the variability and adaptability of moulds make it practically impossible to set some general and stable conditions for their development in food, as well as for the production of mycotoxins. The situation of the meat-processing plants, the slaughter of animals, insufficient cleaning and disinfection of working areas, instruments and other equipment are the most important sources of food contamination by toxicogenic moulds.

As reported by many authors (2, 5, 18,19), numerous strains of microscopic filamentous fungi, isolated from the surface of various meat products, show *in vitro* the ability to produce toxic substances. In our study (Tab. 2, 3) the most frequently isolated mould genera were as follows: *Penicillium* spp., *Mucor* spp., and *Rhizopus* spp. However, some strains of *Aspergillus flavus* and *Fusarium moniliforme,* known as potential producers of mycotoxins, have also been isolated.

Our results are comparable to those reported in the literature (1) referring to the 90 % occurrence of *Penicillium* sp. and the 4 % occurrence of both *Aspergillus* sp. and *Mucor* sp. in raw fermented meat products. The presence of aflatoxin-producing fungi (*Aspergillus flavus* and *Aspergillus oryzae*) was reported in lunch meat (16). An average incidence of 20 % was reported for *Aspergillus flavus* and *Aspergillus parasiticus* in smoked meat products, pork salami, bacon and ham (3). As seen from our results, one must consider that any controllable development of microscopic filamentous fungi in food is undesirable, because it can result in the development of superficial, profound or systemic mycosis, or even mycoallergy.

The presence of moulds in meat products usually causes a decrease in their biological value (due to the enzymatic degradation of meat components). Moulds often come into metabolic interactions with various bacterial pathogens. Thus, they can participate in an outbreak of food-borne illness. These interacions have already been well-documented between moulds and *Clostridium botulinum* or *Staphylococcus aureus* (9). Mould metabolic activity results in the neutralization of organic acids, which is accompanied by an increase in pH-value. Under such conditions, spores of *Clostridium botulinum* are able to germinate and to start the production of botulinotoxin. A less acidic environment also enables the formation of enterotoxins by *Staphylococcus aureus.*

The development of microscopic filamentous fungi in meat products must not be neglected. Moulds must be studied and identified permanently. Food producers must follow the principles of good manufacturing practice and take preventive measures in order to reduce the growth of microscopic filamentous fungi and the production of their toxic metabolites in finished products (4).

REFERENCES

1. Andersen, S. J., 1995: Compositional changes in surface mycoflora during ripening of naturally fermented sausages. *J. Food Protec.,* 58, 426—429.

2. Berwal, J. S., Dinchev, D., 1991: Molds are protective cultures of raw dry sausages. *J. Food Prot.,* 58, 817—819.

3. Cvetnic Z., Pepeljnjak, S., 1995: Aflatoxin-producing potential of *Aspergillus flavus* and *Aspergillus parasiticus* isolated from smoked-dried meat. *Die Nahrung,* 39, 302—307.

4. Čonková, E., Para, Ľ., Kočišová, A., 1993: The growth inhibition of some microscopic filamentous fungi by selected organic acids (In Slovak). *Vet. Med.-Czech,* 38, 723—727.

5. Jesenská, Z., 1987: Microscopic *Filamentous Fungi* in *Food-and Feedstuffs* (In Slovak). Alfa, Bratislava, 318 pp.

6. Leistner, L., 1986: Mold-ripened foods. *Fleischwirtschaft,* 66, 1385—1388.

7. Ostrý, V., 2001: The occurrence of moulds in meat and meat products (I) (In Czech). *Maso,* 5, 20—24.

8. Ostrý,V., Ruprich, J., 2001: The occurrence of moulds in meat and meat products (II) (How is the topical status in The Czech Republic) (In Czech). *Hygiena,* No. 6, 17—21.

9. Polster, M., Hartlová, D., Králiková, I., 1985: Frequency of the occurrence of *Aspergillus flavus* aflatoxicogenic variants in the environment of food-processing establishments (In Czech). *Čs. hyg.,* 9, 442—446.

10. The Slovak *Codex Alimentarius* (In Slovak), 1998: Štvrtá hlava: Mikrobiologické požiadavky na potraviny, kozmetické prostriedky a obaly na ich balenie. Výnos ministerstva pôdohospodárstva SR a Ministerstva zdravotníctva SR zo 16. decembra 1997 č. 557/1998-100, ktorým sa vydáva prvá časť, druhá a tretia hlava druhej časti Potravinového kódexu SR, XXX.

11. Steinhauser, L. *et al.*, 1995: *Meat Hygiene and Technology* (In Czech). LAST, Brno, 15—108.

12. STN ISO 560102, 1997: *Mikrobiológia. Všeobecné pokyny pre prípravu riedení pri mikrobiologickom skúšaní (In Slovak). Slovenský ústav technickej normalizácie, Bratislava.*

13. STN ISO 4833, 1997: *Mikrobiológia. Všeobecné pokyny na stanovenie celkového počtu mikroorganizmov (In Slovak). Slovenský ústav technickej normalizácie, Bratislava.*

14. STN ISO 4832, 1997: *Mikrobiólogia. Všeobecné pokyny na stanovenie počtu koliformných baktérií (In Slovak). Slovenský ústav technickej normalizácie, Bratislava.*

15. STN ISO 6888, 1997: *Mikrobiológia. Všeobecné pokyny na stanovenie počtu baktérií Staphylococcus aureus* (In Slovak). Slovenský ústav technickej normalizácie, Bratislava.

16. STN ISO 6579, 1997: *Mikrobiológia. Všeobecné pokyny pre metódy a dôkaz baktérií rodu Salmonella* (In Slovak). Slovenský ústav technickej normalizácie, Bratislava.

17. STN ISO 7954, 1997: *Mikrobiológia. Všeobecné pokyny na stanovenie počtu kvasiniek a plesní (In Slovak). Slovenský ústav technickej normalizácie, Bratislava.*

18. Zaky, Z. M., Ismail, M. A., Refaire, R. S., 1995: *Aspergillus flavus* and aflatoxins residues in luncheon meat. *Assiut Vet. Med.,* 33, 114—118.

19. Wu, M. T., Ayres, J. C., Koehler, P. N., 1974: Toxigenic *Aspergilli* and *Penicillia* isolated from aged, cured meats. *Appl. Microbiol.,* 28, 1094—1096.

Received July 3, 2003

FOLIA VETERINARIA, 47, 4: 221—224, 2003

THE EMERGENCY SLAUGHTER OF HORSES — CAUSES AND TRENDS IN FUTURE DEVELOPMENT

Kozák, A.[1], Večerek, V.[2], Bartošek, B.[2]
Chloupek, P.[2], Bedáňová, I.[2]

[1]Veterinary Administration of the City of Prague, Na Kozačce 3, 120 00 Prague 2
[2] Veterinary and Pharmaceutical Sciences University of Brno
Palackého 1-3, 612 42 Brno0
The Czech Republic

ovs.praha_mesto@icsvscr.cz

ABSTRACT

The present work is focussed on the determination of the most frequent causes of emergency slaughter of horses and the assessment of future trends. The causes of emergency slaughter in the Czech Republic from 1997 to 2002 have been divided into the following groups: infectious diseases, respiratory diseases, digestive diseases, musculoskeletal diseases, complications *post partum* and diseases of miscellaneous aetiology (disposal of breeding, low performance, cachexy, decrepitude, injuries, etc.). The trends of future development have been determined as an index equal to the ratio of the relative occurrence of the findings during the period 2000 to 2002 to the same figures from the period 1997 to 1999. A very high occurrence of musculoskeletal diseases has been found (46.62 %) showing a stable trend in the long-term (index 1.06), where no significant increase or decrease was demonstrated. Furthermore, the occurrence of the diseases of miscellaneous aetiology was also high (34.84 %), with similarly stable trend (index 0.99) lacking any significant increase or decrease. The cases of emergency slaughter were reflected in the total number of condemnations in equine carcasses (18.96 %) with a long-term trend to increase (index 1.44), which has been confirmed as highly significant. The results confirmed that emergency slaughter of horses was mainly due to musculoskeletal diseases which lead to the culling of horses that cannot be used for sport or work any more. The results also reflected the fact that the utilisation of horses as a food source is of a very low priority.

Key words: condemnation of carcasses; emergency slaughters; findings at slaughterhouses horses

INTRODUCTION

The emergency slaughter of horses indicates the burden on different organs caused by utilisation and transport of horses. Such a burden may result in the deterioration in the health of the animals. The causes of emergency slaughter are specified on the basis of findings during the inspection of meat and organs after slaughter. Kofer *et al.* (1) have emphasised the importance of evaluating findings obtained during the inspection of meat and organs in slaughterhouses. These inspection activities after slaughter result in the classification of carcasses as capable for human consumption (edible), capable for processing (conditionally edible) and condemned.

Kozak et al. (2) have studied the occurrence of emergency slaughter in horses. The authors have reported a long-term decrease in the relative numbers of emergency slaughter from 86.09 % to 27.91 % when comparing the periods 1989 to1994 and 1995 to 2000.

No information about the causes of emergency slaughter in horses has been published. Some related works have only analysed selected *post mortem* changes in the horses slaughtered at regular slaughterhouses. For instance Ley (4) has studied the values of pH and meat temperature in slaughtered horses and a donkey and evaluated the degree of bleeding and ability of meat to bind water.

Kozak *et al.* (3) have studied the occurrence of edible, conditionally edible and condemned carcasses of slaughter animals. The authors have reported a decrease in the occurrence of condemned equine carcasses in the Czech Republic from 10.81 % to 5.01 % when comparing the periods of 1989 to1994 and 1995 to 2000.

The aim of the present work is to determine the most frequent causes of emergency slaughter in horses and trends in their future development. Furthermore, the impact of emergency slaughter on the decisions for the condemnation of equine car-

casses is also evaluated. A long-term trend in the development of numbers of condemned equine carcasses is identified. The results are important for the determination of the chief burdens on equine organs with regard to their utilisation and transport.

MATERIAL AND METHODS

The emergency slaughter of horses during the period 1997 to 2002 has been studied. The findings have been divided into the following groups: infectious diseases, respiratory diseases, digestive diseases, musculoskeletal diseases, complications *post partum* and diseases of miscellaneous aetiology (disposal of breeding, low performance, cachexy, decrepitude, injuries, etc.). Subsequently, the decision of the official veterinarian on the classification of carcasses after emergency slaughter has been recorded as follows: edible, conditionally edible and condemned.

The frequency in the absolute and relative figures for the different causes of emergency slaughters and the classification into the three groups, as above, have been determined for individual years over the whole period of monitoring from 1997 to 2002. Furthermore, the values of the absolute and relative frequency of emergency slaughter have also been determined as well as the results of carcass classification for the whole period 1997 to 2002. The periods of 1997 to 1999 and 2000 to 2002 have been evaluated separately in order to assess the trends in the development of the causes of emergency slaughter.

Both periods have been compared by means of an index calculated as a ratio of relative frequencies 2000 to 2002 in contrast with 1997 to 1999. An index greater than 1.00 suggests an increasing trend in the occurrence of the respective cause of emergency slaughter or classification in the respective group based on the comparison of both these periods. An index equal to 1.00 indicates that no increase in the frequency of the respective cause of emergency slaughter or classification in the respective group occurred. In cases when the value of the index was calculated as smaller than 1.00 a decreasing trend in the relative frequencies for the respective cause of emergency slaughter or classification in the respective group is demonstrated in the comparison of these periods. The statistical significance of increasing or decreasing trends was determined by statistical software Unistat (Unistat Statistical Package, Unistat Ltd.), using a module for the calculation of relative frequencies.

RESULTS

Table 1 presents the frequency in absolute figures of the different causes of emergency slaughter of horses and the results of carcass classification after slaughterhouse inspection. The figures cover individual years from the period of 1997 to 2002. Table 2 presents the same figures as Table 1 expressed in relative values (in %). Table 3 presents the causes of emergency slaughter and the results of carcass classification after slaughterhouse inspection in absolute and relative figures for the whole period 1997 to 2002 and, furthermore, also separately for the periods of

Table 1. The breakdown of causes of emergency slaughter in horses in absolute figures by individual years

Year Causes of emergency slaughter	1997 frequency	1998 frequency	1999 frequency	2000 frequency	2001 frequency	2002 frequency
Infectious diseases	0	0	0	0	0	0
Respiratory diseases	11	22	13	5	8	12
Digestive system diseases	27	29	30	23	19	8
Musculoskeletal diseases	108	124	98	101	74	53
Complications *post partum*	5	2	2	1	4	1
Miscellaneous aetiology	88	89	77	70	54	39
Total	239	266	220	200	159	113
Condemned	29	46	42	41	42	27
Conditionally edible	70	59	67	78	73	40
Edible	140	161	111	81	44	46

Table 2. The breakdown of causes of emergency slaughter in horses in relative figures by individual years

Year Causes of emergency slaughter	1997 %	1998 %	1999 %	2000 %	2001 %	2002 %
Infectious diseases	0.00	0.00	0.00	0.00	0.00	0.00
Respiratory diseases	4.60	8.27	5.91	2.50	5.03	10.62
Digestive system diseases	11.30	10.90	13.64	11.50	11.95	7.08
Musculoskeletal diseases	45.19	46.62	44.55	50.50	46.54	46.90
Complications *post partum*	2.09	0.75	0.91	0.50	2.52	0.88
Miscellaneous aetiology	36.82	33.46	35.00	35.00	33.96	34.51
Total	100.00	100.00	100.00	100.00	100.00	100.00
Condemned	12.13	17.29	19.09	20.50	26.42	23.89
Conditionally edible	29.29	22.18	30.45	39.00	45.91	35.40
Edible	58.58	60.53	50.45	40.50	27.67	40.71

Table 3. The causes of emergency slaughter of horses and trends in their development

Causes of emergency slaughter	1997–2002		First period 1997–1999		Second period 2000–2002		Trend	Signif.
	frequency	%	frequency	A %	frequency	B %	B/A index	P
Infectious diseases	0	0.00	0	0.00	0	0.00	0.00	1.00
Respiratory diseases	71	5.93	46	6.34	25	5.30	0.83	0.45
Digestive system diseases	136	11.36	86	11.86	50	10.59	0.89	0.50
Musculoskeletal diseases	558	46.62	330	45.52	228	48.31	1.06	0.34
Complications *post partum*	15	1.25	9	1.24	6	1.27	1.02	0.96
Miscellaneous aetiology	417	34.84	254	35.03	163	34.53	0.99	0.86
Total	1197	100.00	725	100.00	472	100.00	1.00	
Condemned	227	18.96	117	16.14	110	23.31	1.44	**0.00
Conditionally edible	387	32.33	196	27.03	191	40.47	1.50	**0.00
Edible	583	48.71	412	56.83	171	36.23	0.64	**0.00

Explanations: ** — a highly significant difference was found between the first and the second period ($P \leq 0.01$)

1997 to 1999 and 2000 to 2002 for horses. The trends of their development in the long term expressed as indices of the increase or decrease in the relative frequency of different causes of emergency slaughter and results of carcass classification after slaughterhouse inspection are shown in the same Table as well.

A very high occurrence of musculoskeletal diseases was found (46.62 %) showing a stable trend in the long-term (index 1.06), where no significant increase or decrease was demonstrated. Furthermore, the occurrence of the diseases of miscellaneous aetiology was also high (34.84 %), with a similar stable trend (index 0.99) lacking any significant increase or decrease. The cases of emergency slaughter were reflected in the total number of condemnations in equine carcasses (18.96 %) with a long-term trend to increase (index 1.44), which has been confirmed as highly significant.

DISCUSSION

The causes of emergency slaughter in horses have suggested the types of burden to which the horses are mostly exposed in the course of their utilisation and transport and which have caused health disorders resulting in emergency slaughter. These causes are subsequently reflected in the results of carcass classification after slaughterhouse inspection. In the long term the numbers in emergency slaughter of horses have been decreasing, which supports the trend published by Kozak *et al.* (2). We have found out that the most frequent causes of emergency slaughter for horses are musculoskeletal diseases and diseases of miscellaneous aetiology. The frequency of the diseases from the former group has been, however, particularly high and accounts for almost one half of all emergency slaughter.

Emergency slaughter due to musculoskeletal diseases is a cause specific to horses. It is related to the strong burden on the musculoskeletal system due to the utilisation of horses for sport or work. The results cannot be compared to data from the literature, because no other similar studies are available. The only related works are those focusing on selected parameters of the meat of slaughtered horses — for instance Ley (4). However, no analysis of emergency slaughter has been presented in these publications.

The carcasses of approximately one fifth of all horses after emergency slaughter were condemned. This value was relatively low due to the causes of emergency slaughter. The primary reasons were usually injuries and damage to the musculoskeletal system which by no means resulted in the necessity to condemn the carcasses.

Nevertheless, the numbers of condemned equine carcasses after emergency slaughter has increased in the long term. The numbers of emergency slaughter, however, show a trend different to the one observed by Kozak *et al.* (3) in cases of slaughter in regular slaughterhouses. These results can be explained by the fact that, in general, the health of horses had improved in the long term, which resulted in the decrease of numbers of condemned equine carcasses at regular slaughterhouses. On the other hand, in cases of individual horses that suffered from a disease or injury which subsequently led to a decision for emergency slaughter, it has to be noted that the utilisation of horses as food animals is of a very low priority. Therefore the animals are not rapidly culled and when they get to a slaughterhouse the disease is usually so advanced that the carcass can be only condemned.

CONCLUSION

The results demonstrate that in the long term horses are exposed to significant burdens affecting the musculoskeletal system or causing diseases of miscellaneous aetiology. Mus-

culoskeletal diseases and diseases of miscellaneous aetiology as causes of emergency slaughter in horses do not show an increasing trend. These diseases influenced the condemnation of equine carcasses after emergency slaughter. The results show that emergency slaughter of horses are mostly due to musculoskeletal diseases resulting in culling animals that cannot be further utilised for sport or work. Another factor that influences the results is that the utilisation of horses as food animals is in general of a very low priority. The increase in the absolute numbers of condemned equine carcasses was considered negative.

Acknowledgments

This paper was prepared as a part of Research Project of the Ministry of Education, Youth and Sports of The Czech Republic No. 16270005 *"Research of Current Hygienic Aspects of Production of Food and Raw Materials of Animal Origin With Regard to Their Safety."*

REFERENCES

1. Kofer, J., Kutschera, G., Fuchs, K., 2001: Monitoring of animal health at abattoirs. *Fleischwirtschaft,* 81, 107—111.

2. Kozák, A., Večerek, V., Steinhauserová, I., Chloupek, P., Pištěková, V., 2002: The occurrence of emergency slaughters in selected species of food animals. *Folia Veterinaria,* 46, 131—134.

3. Kozák, A., Večerek, V., Steinhauserová, I., Chloupek, P., Pištěková, V., 2002: Results of slaughterhouse carcass classification (capable for human consumption, capable for processing and condemned) in selected species of food animals. *Vet. Med.-Czech,* 47, 26—31.

4. Ley, T., 1997: Investigations on postmortal changes in carcasses of horses. *Fleischwirtschaft,* 77, 172—175.

Received June 13, 2003