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FOLIA VETERINARIA, 50, 1: 3—4, 2006

FOLIA VETERINARIA (1956—2006)

The anniversary of the 55-year existence of our alma mater (in 2004) touched all areas of its life, including its scientific periodical – *Folia Veterinaria*. In that year the 48th Volume of this Journal had already appeared issued because its first Volume was published in 1956, seven years after the establishment of the Veterinary College in Košice. In 2006 this Journal celebrates the semi-centennial of its existence.

What has caused this apparently considerable delay in issuing *Folia veterinaria*? Personal observers of the process of building of the first veterinary education institution in Slovakia and information provided in the jubilee publication *"55 years of the University of Veterinary Medicine"* (1949—2004) – UVM in Košice, 2004 – explained the situation clearly. Fifty-seven years ago the Veterinary College in Košice was built "on a green meadow". Because of this the young teaching staff which formed "while marching", had to concentrate on facilities where individual disciplines had to be taught and on teaching itself. The success in training new veterinary professionals in Slovakia was documented by the first graduation of 62 veterinary surgeons in the Fall of 1954.

At that time the research activities of the staff had reached a stage of full development. This is witnessed by papers published chiefly in the nationwide journal *Veterinářství* (its first issue was published in 1951) and in *Veterinársky zborník* (founded in 1952) published in Bratislava, renamed later as *Veterinársky časopis*. Prof. MVDr. Ján Hovorka, the first rector of the Veterinary College in Košice was the Editor-in-Chief of both Slovak journals:

List of published papers: Bartík, M., Zwick, K.: Polarography of blood sera of farm animals based on Brdička reaction (In Slovak). *Vet. zborník* 1, 1952, 40—43; Breza, M.: Poisoning of farm animals by fluorine compounds (In Slovak), *Veterinářství* 6, 1952, 62–63; Zvick, K.:Study of bond of formaldehyde with proteins (Determination of formaldehyde in proteinaceous environment) (In Slovak), *Vet. časopis* 2, 1953, 139—146; Káldy, A.: Experiment with feeding ricinus oil leavings to sheep (In Slovak), *Vet. Čas.* 2, 1953, 241—254; Vodrážka, J.: On the use of estrogenous substances in veterinary medicine (In Slovak), *Veterinářství* 3, 1953, 193—197; Sokol, A., Rosocha, J., Špeník, M: Comparative study of the retardative effect of antireticular-cytotoxic and antineural-cytotoxic serum on the development of infectious process at porcine encephalomyelitis (In Slovak), *Vet. zborník* 1, 1952, 10—27; Sokol, A., Rosocha, J., Špeník, M: Additional study of immunogenesis, pathogenesis and pathology of porcine encephalomyelitis (In Slovak), *Vet. časopis* 2, 1953, 201—216; Hovorka, J.: Epizootological survey of equine strongylids and *Parascaris equorum* in basins of east Slovakian rivers and their relationship to colics (In Slovak), *Vet. zborník* 1, 1952, 51—78.

During the fifty years of publishing the *Folia veterinaria* journal there were many changes in editors, editorial boards and contributors. The first two Volumes (1956 and 1957/1958) were prepared by Doc. MVDr. František Hrudka and MVDr. Michal Breza – who held the posts of chief and executive editors. After that, before issuing the 3rd Volume, these posts were taken over by dep. Doc. MVDr. Jaroslav Oto Vrtiak and MVDr. Ľudovít Slanina. Later on, members of senior academic personnel held alternatively the posts of chief and executive editors of *Folia veterinaria*. One exception that should be mentioned was the long-term involvement of Prof. MVDr. Jozef Arendarčik, DrSc., who held the post of executive editor for a number of years. Since 1993 (Volume 37), these two posts were supplemented with by post of technical editor. This post has been held by MVDr. Michal Breza, CSc., who has retained it even after retiring in 1993.

Up to the 25th Volume (1981) of *Folia veterinaria*, the contributions were published only in Slovak. However, the summaries were in foreign languages, namely in Russian, German and later also in English. Even in this form the papers raised interest abroad, which is witnessed by many citations in papers published in this field at home and abroad. Starting from the 8th Volume (1964), the original form of annual collected papers was replaced by individual issues (1—4) or double issues (1—2, 3—4).

Starting from the eighties, along with publishing scientific and specialised papers, the journal provided also information about university happenings in the teaching and scientific-research area and about important social events. Moreover, the history of the quarter-centennial existence of the Veterinary College in Košice was summarised in a Supplement to the 18th Volume

of *Folia veterinaria* (1974), which also supplemented the first two "decennials" issued as individual publications (edited by late Prof. MVDr. Karol Fried). He also edited another Supplement, issued in 1984 (a Supplement to the 30th anniversary of the existence of the establishment which, although delayed, is an addition to the Supplement from 1974 and provides additional details about the life and work of college staff in the period 1974—1979).

A very important milestone is the 26th Volume of *Folia veterinaria* (1982), because starting with this volume all papers and contributions were published in English. An exception are the two mentioned Supplements, published in 1974 and 1984 in Slovak on the occasion of the 25th and 30th years existence of the Veterinary College (VŠV) in Košice.

The new social conditions in Europe after 1989, and the establishment of the independent Slovak Republic (1993) called for closer collaboration particularly with partners from West European countries. Therefore the management of UVM and the Editorial Office of *Folia veterinaria* took some important measures regarding publication of this journal. Favourable conditions for their intention developed particularly after the opening of a new programme of study at UVM (starting from 1991) – General veterinary medicine for foreign students taught in English language. The presence of English speaking lectors – natives of the United Kingdom and the USA – allowed the university to raise the language level of *Folia veterinaria* (language revision of papers). Starting from the 37th Volume (1993), the journal changed its format (A4) to correspond to that of worldwide scientific journals. Selections of papers helped to increase citations in journals published abroad. Starting from the 42nd Volume (1998), the number of papers published annually has increased considerably (doubled) after returning to four individual issues per year, each to an extent of 56 printed pages.

In addition to four compulsory annual issues of *Folia veterinaria* the following additional Supplements have been published since 1998:

In 1998 (42nd Volume):

AVIAN MICROGRAVITY
Proceedings of the Third Symposium held
on September 29 – October 2, 1997
Košice, The Slovak Republic
Volume V
"Current Trends in Cosmic Biology and Medicine"
Edited by K. Boďa and T. S. Guryeva

In 2001 (45th Volume, No. 1):

AVIAN MICROGRAVITY
Proceedings of the International Symposium QUAIL SK 6,
held between the 28th and 30th November, 2000 in Košice,
The Slovak Republic
Volume VI
"Current Trends in Cosmic Biology and Medicine"
Edited by K. Boďa, T. S. Guryeva, and V. Sabo

In 2002 (46th Volume, No. 2):

ECOLOGY AND VETERINARY MEDICINE V
"HYGIENIC AND ECOLOGICAL PROBLEMS IN RELATION TO VETERINARY MEDICINE"
International scientific conference
UVM Košice, The Slovak Republic
September 18—19, 2002

In 2004 (48th Volume, No. 1):

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

In 2005 (49th Volume, No. 3):

ECOLOGY AND VETERINARY MEDICINE VI
"HYGIENE AND ECOLOGICAL PROBLEMS IN RELATION TO VETERINARY MEDICINE"
International scientific conference
UVM Košice
The Slovak Republic
(June 16—17, 2005)

In addition to its representational role – publication of results of the academic staff of UVM in Košice and professionals from other workplaces at home and abroad dealing with similar issues – the *Folia veterinaria* journal has been since its establishment an effective tool for augmentation of the fund of the Institute of Scientific Information and Library of UVM in Košice. At the present, through mutual exchange for *Folia veterinaria*, our University obtains almost 70 foreign scientific and specialised journals.

In conclusion we can state that by its semi-centennial existence, the journal *Folia veterinaria* has contributed considerably to the excellent worldwide image of the former *Veterinary College*, the present *University of Veterinary Medicine* in Košice, which in 1999 could proudly point to its successful, longer than semi-centennial teaching and scientific activities.

Dr. h. c. Prof. Rudolf Cabadaj, DVM, PhD
Rector of UVM and Editor in Chief

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THE EFFECT OF HORMONAL STIMULATION ON CATECHOLAMINE AND MONOAMINE OXIDASE ACTIVITY IN THE PITUITARY GLAND OF EWES

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ABSTRACT

The effect of hormonal stimulation on catecholamine levels and activity of its degradation enzyme monoaminooxidase in the hypophysis of ewes in the oestric period was studied by the radioenzymatic method. Monoaminooxidase activity was determined radiochemically. The oestrus of ewes was synchronized with Agelin sponges (Agelin, Spofa, Ivanovice on Hana, Czech Republic) containing 20 µg chlorosuperlutin. After completed synchronization we induced superovulation in the experimental group by means of 1500 IU serum gonadotrophin (SG, Ivanovice on Hana, Czech Republic). The results indicate that hormonal serum gonadotrophin stimulation increases ($P < 0.01$) the pituitary dopamine and epinephrine levels in ewes significantly. In comparison with the control group norepinephrine concentration did not change in this tissue. MAO activity in the hypophysis decreased significantly to almost one half in comparison with control values ($P < 0.001$). According to our results, the serum gonadotrophin in combination with hyperestrogenization influences dopamine and norepinephrine metabolism in the hypophysis of hormonally stimulated ewes and reduces monoaminooxidase activity selectively.

Key words: dopamine; epinephrine; ewes; hormonal preparations; monoaminooxidase; norepinephrine; pituitary gland; superovulation

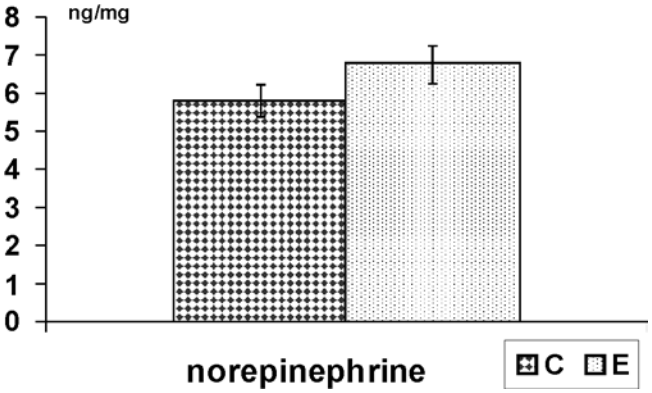
INTRODUCTION

At the begininnig of the eighties Björklund *et al.* (4) and Saavedra *et al.* (19) demonstrated the presence of catecholamines in the adenohypophyseal neurointermedial lobe in rats. Later it was found that for catecholamines present in the hypophysis the greatest part is formed by dopamine. Norepinephrine and epinephrine occur in the hypophysis in lower concentrations (7, 2). The dopaminergic tuberoinfundibular system innervates the area *pars intermedia* and *lobus posterior* of the hypophysis with neural projections.

The functional nuclei of this system are in the hypothalamus in the area *n. arcuatus* and *n. paraventricularis* and its axons project through the *eminentia mediana*, around the endocrine cells in *pars intermedia* and in the pericapillary spaces in the hypophysis (3, 4). The function of dopamine in the pituitary *lobus posterior* has been defined as a neuromediator or neurohormone or combination of the two (7).

There is evidence that dopamine participates in the regulation of water supplies in the organism and in the secretion of oxytocin. During lactation the content of pituitary dopamine decreases and its turnover increases. Hypothalamic dopamine participates in the regulation of prolactin secretion (PIH) in that it is released from the *eminentia mediana* into the portal blood vessels and acts as important factor in prolactin secretion. In the *pars intermedia* of the adenohypophysis dopaminergic and adrenergic receptors also participate in regulating the release of the melanotropic stimulating hormone.

Since there is evidence that estrogens also participate largely in metabolic and functional catecholamine changes in the rat



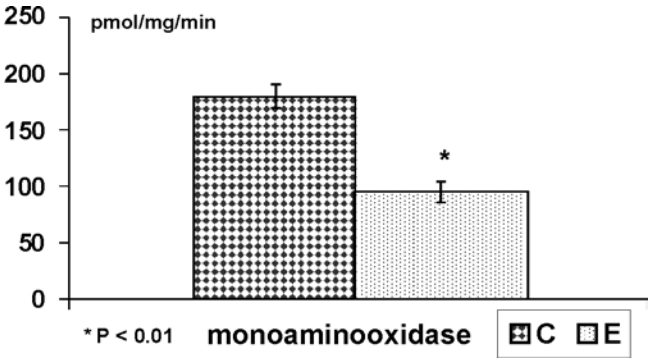
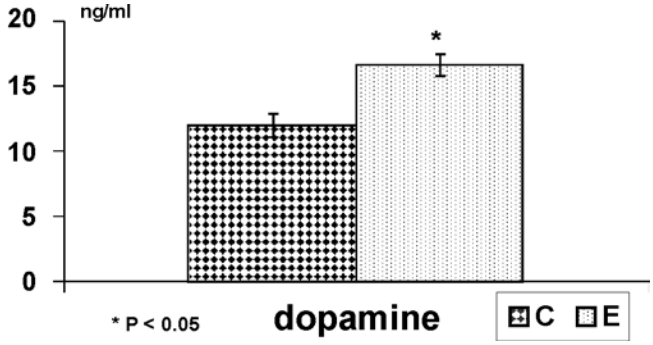


Fig. 1. Effect of serum gonadotrophin hormonal stimulation on pituitary norepinephrine and epinephrine levels in ewes. Results are expressed in ng.mg^{-1} protein, arithmetic means ± S.E.M.

C — control group with synchronised oestrus,
Agelin sponges containing 20 mg chlorsuperlutin
E — experimental group with synchronised oestrus which was given 1500 IU serum gonadotrophin



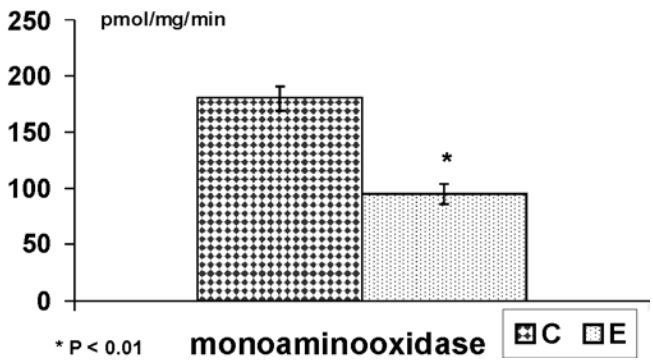


Fig. 2. Effect of synchronisation and application of serum gonadotrophin on pituitary dopamine and on monoaminooxidase activity in the hypophysis (in pmol MAO produced by mg proteins per minute). C, E — see Fig. 1

hypophysis (19) we focused on the effect of the superovulation preparation of serum gonadotrophin, which hyperestrogenises dopamine, norepinephrine and epinephrine levels, and on the activity of their degradation enzyme in the pituitary gland of ewes during the oestric period.

MATERIAL AND METHODS

In our study we used 10 ewes of the Wallachian breed, aged 3 to 4 years, with a mean weight 41 ± 6 kg in their oestric period. The oestrus of all ewes was synchronized with Agelin sponges (Agelin, Spofa) containing 20 mg chlorsuperlutin, which we installed for a period of 12 days. The first group, numbering 4, acted as a control group. The ewes in the second group (6) were, after the completed synchronization of the oestrus (on the 12th day of the study), hormonally stimulated by the administration of 1500 IU of serum gonadotrophin (Ivanovice on Hana, Czech Republic). The animals were bled to death 104 hours after receiving serum gonadotrophin (SG), we rapidly removed the pituitary gland and kept it in a frozen state in in liquid nitrogen until further processing.

The tissue was homogenized in cold in Potter–Elvehjem homogenisers in a deproteinisation solution containing $HClO_4$ ($c = 0.4\ mol^{-1}$) and reduced glutathion ($c = 0.05\ mol^{-1}$) at an amount of 10 µl per 1 mg tissue. The tissue homogenates were centrifuged at 10,000 g.min^{-1} for thirty minutes. Catecholamines (norepinephrine, dopamine and epinephrine) were determined radioenzymatically using the Catechola test (Adico, Praha, Czech Republic). S-adenosyl-l-methyl-3H-methionine served as substrate. After isolation of the catecholamines their individual radioactive derivates were distributed chromatographically on Silufol UV 254 disks. The activity of 3H catecholamine derivates was measured on scintillation spectrometry Packard-Tri-Carb. Results are expressed in catecholamine ng.mg^{-1} proteins. Proteins were determined in identical homogenate tissues according to Lowry *et al.* (12). The activity of monoaminooxidase was estimated in tissue homogenates radiochemically according to Wurtman and Axelrodt (22). As substrate we used 14C -tryptamine of specific activity $18.5.10^{-7}$ Bq.nmol^{-1}, using for one sample 6.25 nmol 14C-tryptamine. Results were expressed in pmol MAO produced by 1 milligram protein per minute and statistically evaluated by Student's *t*-test.

RESULTS

Catecholamine concentrations in the pituitary gland of the control ewes, determined by the radioenzymatic method, are shown in Figs. 1 and 2. The greatest concen-

tration was achieved by dopamine (12.05 ± 0.121 ng.mg^{-1}), norepinephrine and epinephrine levels were lower.

Application of 1500 IU serum gonadotrophin induced no changes in pituitary norepinephrine levels (Fig. 1) in comparison with control values. Dopamine concentrations after SG stimulation increased significantly by 37 % ($P < 0.01$). Pituitary epinephrine levels rose from 5.01 ± 0.023 to 7.81 ± 0.052 ng.mg^{-1} and were statistically significant ($P < 0.01$). The activity of monoaminooxidase decreased after serum gonadotrophin application to one half of the values ($P < 0.01$), from 205.01 ± 0.123 to 102.32 ± 0.064 pmol.mg.min^{-1} (Fig. 2).

DISCUSSION

Hypothalamic catecholamines participate in the regulation of gonadotrophin hormone secretion in the oestric cycle of animals. Though current science has a lot of information about the functions of monoamines related to reproduction (8, 15) the effect of hormonal preparations, used for inducing superovulation in farm animals, on the catecholaminergic system of hypothalamus and hypophysis is not fully understood.

The extrahypophyseal hormone SG, which shows LH and FSH activity, has a long half-life of biological degradation in the organism and its application is associated with hyperestrogenization (16). High oestrogen levels have a specific impact on hypothalamic adrenergic receptors and influence catecholamine levels and functions.

Some authors (17, 6, 13) have found after the application of estrogens to ovariectomized rats a reduced norepinephrine turnover in hypothalamic nuclei and subsequent LH decrease in blood plasma although the activity of tyrosin-ß-hydroxylase turnover of dopamine in hypothalamus increased. Barden *et al.* (2) have noted after estrogen application a higher secretion of oxytocin and vasopressin, a rise in hypophyseal dopamine levels in rats and an elevated dopamine turnover in hypothalamus, especially in the *eminentia medialis*. Anatomic and functional interactions between estrogens and catecholamines occur also in the pituitary gland. Saavedra *et al.* (19) have observed after the application of 17-ß-estradiol to rats changes in pituitary catecholamine level balance and an increase in dopamine turnover, particularly in the pars intermedia of hypophysis.

In agreement with these reports in the literature we have observed following SG application a significant rise in dopamine concentration (by 37 %) in the pituitary gland. Similar significant changes occurred in pituitary epinephrine levels ($P < 0.01$). Norepinephrine concentrations were unchanged in comparison with control values. In our previous studies (14, 15) we have found after application of hormonal SG significant changes in catecholamines, especially in the *area preoptica* and *eminentia medialis* of the hypothalamus. After immunological blocking of circulating SG molecules by means of the preparation Antisergon (Antisergon, Spofa) suppressing the hypoestrogenization effect of SG, changes in hypothalamic catecholamine levels were not as pronounced (21, 20).

The elevated pituitary dopamine levels after hyperestrogenization due to SG application, observed in our study on ewes, may by connected with the findings of other authors (10,1) that circulating high estrogen levels condition also changes in tuberoinfundibular activity in the dopaminergic system. Dopamine is released from tuberoinfundibular nerve endings into pituitary portal blood and inhibits prolactin secretion probably via D2 receptors present in plasma membranes of pituitary lactotropic cells. Participating also in this process are estrogens and progesterone, which affect prolactin release into peripheral circulation and modulate dopamine secretion to hypophyseal portal blood.

In estimating the relationship of lactotropic cells to estrogen it may be assumed that estrogens stimulate prolactin secretion and desensitize the pituitary frontal lobe to the inhibitory effect of dopamine (9). Other authors (17) have found that after an increase of dopaminergic activity there occurs a simultaneously modified secretion of the melanotropin stimulating hormone because decreased secretion of this hormone was noted following stimulation of D2 receptors.

Levels of catecholamine degradation enzyme – monoaminooxidase in the hypophysis of ewes are relatively low in comparison with other nerve tissues. It is probable (8, 11) that degradation of pituitary catecholamines by way of MAO takes place to a lesser degree. In spite of that we recorded a marked reduction of MAO after hormonal treatment with SG which correlates with the observed increase of dopamine and epinephrine.

Our results indicate that application of the hormonal SG, routinely used for inducing superovulation in farm animals, selectively influences dopamine and epinephrine metabolism in the hypophysis and simultaneously reduces the activity of their degradation enzyme – monoaminooxidase.

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STUDY OF THE ANTIMICROBIAL ACTIVITY OF TIAMULIN HYDROGEN FUMARATE, AGAINST PATHOGENIC MICROORGANISMS ISOLATED FROM ANIMALS

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SUMMARY

The antibacterial activity of tiamulin hydrogen fumarate, produced by Actavis-JSC, Bulgaria was studied in comparison with tiamulin hydrogen fumarate produced by Clariant-LSM, Italy. Clinical and standard strains of Gram-positive and Gram-negative micro-organisms were tested, utilizing the method of double serial dilutions in geometrical progression on solid and liquid media. Referring to the obtained results of minimal inhibitory concentrations (MIC), it may be concluded, that the MIC of the compared substances are equivalent in terms of their antibacterial activity.

Key words: micro-organisms; minimal inhibitory concentration; tiamulin

INTRODUCTION

Tiamulin is a diterpene antibiotic of expressed activity against a number of Gram-positive (*Streptococci, Staphylococci, Micrococci, Peptococci, Clostridia, Erysipelas* spp., *Listeria, Corynebacteria*) and Gram-negative (*Pasteurella, Shigella, Actinobacillus, Fusobacteria, Campylobacter,* bacteroids) micro-organisms, spirochaetes, mycoplasmas, Actinomycetes, *Leptospira*, etc. (1, 3, 4, 6, 7, 14, 16, 18). Pasteurellas (3) are less susceptible to it, and the species of Enterobacteriaceae (*Salmonella* spp., *Enterobacter, E. coli*), *Bordetella bronchiseptica, Pseudomonas aeruginosa, Proteus* spp. and *Alcaligenes faecalis* (1, 9, 10) are almost resistant.

Tiamulin's high rate of activity against *Brachyspira (Treponema) hyodysenteriae* (the main causative agent of haemorrhagic dysentery in swine) and the accompanying anaerobic intestine flora (*Bacteroides* spp., *Fusiformis necroforus, Clostridium perfringens, Campylobacter coli* (2, 5, 9, 10, 11, 12, 18) is of significance for veterinary medical practice. Its activity against other types of spirochaetes (*Serpulina pilosicoli, S. innocens, S. suis,* etc.) – the causative agents of intestine infections in swine (3, 16, 17) – is also very important.

Attention should be paid to the mycoplasmas (*Mycoplasma gallisepticum, M. hyopneumoniae, M. hyosynoviae, M. arthritidis, M. hyorhinis, M. agalactiae,* etc.) – the causative agents of severe infections in mammals and poultry, which are very susceptible to the action of tiamulin (6, 8, 13).

In 2001 tiamulin hydrogen fumarate was obtained in Balkanpharma from the basidiomycete *Clitopilus pincitus*. For the latter, there is no available data on its antimicrobial activity.

The purpose of this research study is to investigate the antimicrobial activity of tiamulin hydrogen fumarate-Balkanpharma, against the most common pathogenic micro-organisms isolated from animals, and compare it with the activity of an analogous standard product.

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MATERIALS AND METHODS

1. Antibacterial substances

1.1. Tiamulin hydrogen fumarate (operation 01P01) with the activity of 996 IU.mg^{-1}, provided by the Chemical Technologies Unit at the Research and Development (R&D) Department in Actavis JSC, Bulgaria (Certificate of Analysis – 022001);

1.2. Tiamulin hydrogen fumarate, Cod. № 70615, Lot. №1 9801093.0.01, produced by Clariant-LSM, Italia with the activity of 990 IU.mg^{-1} (Certificate of Analysis № OT1202 B01).

2. Bacterial strains

The assay of the compared substances was tested on 155 strains of Gram-positive and Gram-negative micro-organisms, isolated from different animal species (Table 1).

3. Media

Ordinary and selective media for the cultivation of the micro-organisms were used, as follows:

– Columbia agar and broth, Columbia agar with 5 % sheep blood and 0.07 % NAD, Columbia broth with 10 % bovine serum;

– Mueller-Hinton agar and broth, Mueller-Hinton agar and broth with 2 % bovine serum, Mueller-Hinton agar with 5 % sheep blood, heated to 80 °C, Mueller-Hinton broth with 10 % bovine serum;

– Brucella agar with 5 % bovine serum, Brucella broth with 10 % bovine serum;

– Todd-Hewitt agar and broth with 2 % and 5 % bovine serum.

The susceptibility of the above-listed bacteria to the compared substances of tiamulin hydrogen fumarate was determined by the method of double serial dilutions in geometrical progression on solid and liquid media.

As minimal inhibitory concentration (MIC) registered the lowest concentration, in which following cultivation of the seed media at 37 °C for 48 hours, where evident growth of the bacteria studied was not observed. The influence of the quantity of inoculum and the pH of the media on MIC values was considered.

RESULTS

The MIC results, obtained for the tiamulin (TML)-Actavis-JSC, Bulgaria and tiamulin (TML)-Clariant LSM, are summarized in Table 1.

The results enable us to group the utilized micro-organisms, depending on their susceptibility to tiamulin, as follows:

– I. group – micro-organisms with MIC ranging from 0.25 mg.ml^{-1} to 0.50 mg.ml^{-1}: The group includes all strains of *Micrococcus* sp., some strains of *Staph. epidermidis*, and two strains of *Str. pyogenes*, showing no differences in the activity of the compared substances. Tiamulin, produced by Balkanpharma, appears to suppress a greater number of strains from *Micrococcus* (seven strains) in a concentration of 0.25 mg.ml^{-1}, while tiamulin, produced by Clariant-LSM suppresses the growth of the same strains in a concentration of 0,50 mg.ml^{-1}.

– II group – micro-organisms with MIC ranging from 2 mg.ml^{-1} to 8—16 mg.ml^{-1}: Here all strains of *Staphylococci* of the two species, the *Streptococci* from group A, B and C and the strains of *Actinobacillus pleuropneumoniae* and *Bacillus subtilis* can be included.

– III group – micro-organisms with MIC of tiamulin, ranging from 8 mg.ml^{-1} to 64 mg.ml^{-1} – including the strains *Pasteurella multocida*, and to a certain extent, *Bacillus cereus*: In studies of liquid media all tested strains of *Pasteurella* have been suppressed at one dilution lower, with both of the studied substances, compared to the solid media, but in all cases their activity proved to be within similar MIC ranges.

– IV group – includes micro-organisms with high levels of MIC, in our particular case in the range of 128 mg.ml^{-1} to 256 mg.ml^{-1} and above 256 mg.ml^{-1}: It shows that MIC100 of *Streptococcus faecalis* for both products is 256 mg.ml^{-1}. Our studies show that in a concentration of 256 mg.ml^{-1}, tiamulin inhibits the growth of the strains of *E. coli* and *Salmonella*, while the rest of the studied Gram-negative bacteria growth is at 512 mg.ml^{-1}.

DISCUSSION

The comparative studies carried out with tiamulin-Actavis-JSC and tiamulin-Clariant-LSM, show that both of the tested substances possess equivalent antimicrobial activity against pathogenic bacteria, isolated from different animal species. A similar sensitivity of the micro-organisms towards the substances studied is registered, which allows us to divide them into four groups, depending on their susceptibility:

Group I include those bacteria, with the highest sensitivity. The data on the cocci and mycoplasmas are in accordance with the data reported by other authors (1, 4, 5, 6, 8, 11, 13).

Special attention has to be paid to Group II and Group III, demonstrating a susceptibility with an MIC, ranging from 2 μg.ml^{-1} to 64 μg.ml^{-1}, at which level the growth of all *Staphylococcus* strains are inhibited, the obligatory pathogenic streptococci from group A, B and C, as well the representatives from *Actinobacillus*. The same is true for the strains from *Pasteurella* and *Bacillus subtilis*. These are actually the causative agents for almost half of the bacterial diseases of the digestive tract and lungs in the animals.

Group IV–MIC above 128 μg.ml^{-1}—256 μg.ml^{-1} and higher – includes the "harmless" Gram-negative bacteria from Enterobacteriaceae, the non-fermentative bacteria from *Pseudomonas* and *Streptococcus faecalis*. In this particular case the application of tiamulin would be irrational and usefulness.

The data from the studies, which have been carried out, confirm the results obtained by Erich and Lin (5); Aarestrup and Jensen (1). Erich and Lin (5),

Table 1. Comparative studies of the antimicrobial activity of tiamulin-Actavis JSC, Bulgaria and tiamulin-Clariant LSM

No.	Bacterial strain	No.	MIC (mg.ml^{-1})																					
			0.25		0.5		2		4		8		16		32		64		128		256		> 256	
			R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C
1	*Staphylococcus aureus*	18					6	6	12	12														
2	*Staphylococcus epidermidis*	20			2	2	3	3	15	15														
3	*Micrococcus* spp.	11	7	4	4	7																		
4	*Streptococcus piogenes* (gr. A)	10			2	2			4	4	4	4												
5	*Streptococcus* (gr. B&C)	7					3	3	4	4														
6	*Actinobacillus pleuropneumoniae*	5					1	1	2	1		1	2	2										
7	*Streptococcus faecalis* (gr. D) *Enterococcus*	16			2	2	14	14																
8	*Pasteurella multocida*	7									2	1		1	3	3	2	2						
9	*Bacillus subtilis*	2							2	2														
10	*Bacillus cereus*	3													2	2			1	1				
11	*Escherichia coli*	16																			0	0	-	-
12	*Salmonella* spp.	11																			0	0	-	-
13	*Enterobacter aerogenes*	9																			0	0	-	-
14	*Proteus mirabilis*	7																			0	0	-	-
15	*Pseudomonas aeroginosa*	13																			0	0	-	-
	Total No. of strains	155																						

Legend: R — tiamulin, (TML) — Actavis-JSC, C — tiamulin, (TML) — Clariant-LSM

Karlsson *et al.* (10) who also register that tiamulin is not active against the representatives of Enterobacteriaceae and non-fermentative Gram-negative bacteria. It appears that the media, which are utilised in the studies, are of great importance with regard to the ability to replicate the results and the accuracy of the results. These considerably influence the MIC of tiamulin in the *Streptococcus, Pasteurella* and *Actinobacillus*, and the most appropriate for these species are Brucella agar and Brucella broth with an additive of 5 % bovine serum. Upon utilization of these media, the MICs of tiamulin replicate completely.

The density seed of the media is also important. As well as the kind of the media, the density seed of the non-fastidious bacteria (staphylococci, *Pseudomonas*, Enterobacteria and *Bacillus*), are not so significant. However, complete and constant results are obtained at a density of 10^6—10^7 CFU. The media density seed significantly influences the results in the "fastidious" bacteria – titers below 10^4 CFU of *Streptococcus, Pasteurella* and *Actinobacillus* almost do not yield growth in antibiotic media, regardless of the optimum quality of the other conditions. The results, obtained in the utilization of media with a density of 10^5 and 10^6 CFU, can be replicated, and at MIC of 10^7 CFU the replication rate increases. This is to indicate that with these types of bacteria the optimal density in assessing tiamulin activity is 10^5—10^6 CFU.

It appears that the media pH is of importance to tiamulin's MIC in all of the tested microbial species, and in order to achieve replication of the results the media pH should be within 7.0 and 7.4.

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EVALUATION OF SOME PHYSIOLOGICAL AND HAEMATOLOGICAL PARAMETERS IN THE EWE: INFLUENCE OF SHEARING AND SHELTERING

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ABSTRACT

The pattern of several physiological parameters such as body temperature, heart rate, respiratory rate, haematocrit and haemoglobin content, which are closely related to thermoregulatory potential, have been investigated with the purpose of assessing the shearing and sheltering influence on thermoregulation. Thirty Comisana ewes, one-year old, clinically healthy and well-fed yearling, were used. They were divided into three groups, each consisting of 10 subjects, housed in three different shelters: group A was kept in an outdoor pen, group B was kept in a pen equipped with a shady net (80 % shading) and group C was kept in an outdoor sheepfold with an attached paddock. Through simultaneous monitoring equipment and blood collection by means of jugular venipuncture, some physiological and haematological parameters were assessed for each subject on the following experimental conditions: before shearing, 1st, 2nd, 3rd, 4th and 5th day after shearing. The paired and unpaired *t*-test was applied to determine statistical significances within each group and between groups respectively. Statistically significant differences were observed for all the studied parameters. These results suggest that shearing and sheltering induce adaptative responses in the organism; more specifically, obtained results underline that a shading net provides a more comfortable microclimatic environment to sheep.

Key words: ewe; haematological parameters; physiological parameters; shearing; sheltering

INTRODUCTION

Sheep are mainly exposed to environmental factors (climatic and dietary) variability and to their interaction. It is well known that to maintain a constant body temperature, an animal has to satisfy the condition of "stationary equilibrium", in which the metabolic production of heat is equal to its loss. The thermoneutral zone has been defined as "The range of ambient temperature within which metabolic rate is at a minimum, and within temperature regulation is achieved by non-evaporative physical processes alone" (4). The breadth of the thermoneutral zone depends on age, species and breed, level of nutrition and fleece (17). The breadth of the thermoneutral zone which is significantly correlated to fleece length in the sheep (6) presents a lower critical temperature (LCT) of –4 °C (16) which increases to 28 °C after shearing (14). At an environmental temperature lower than body temperature, heat

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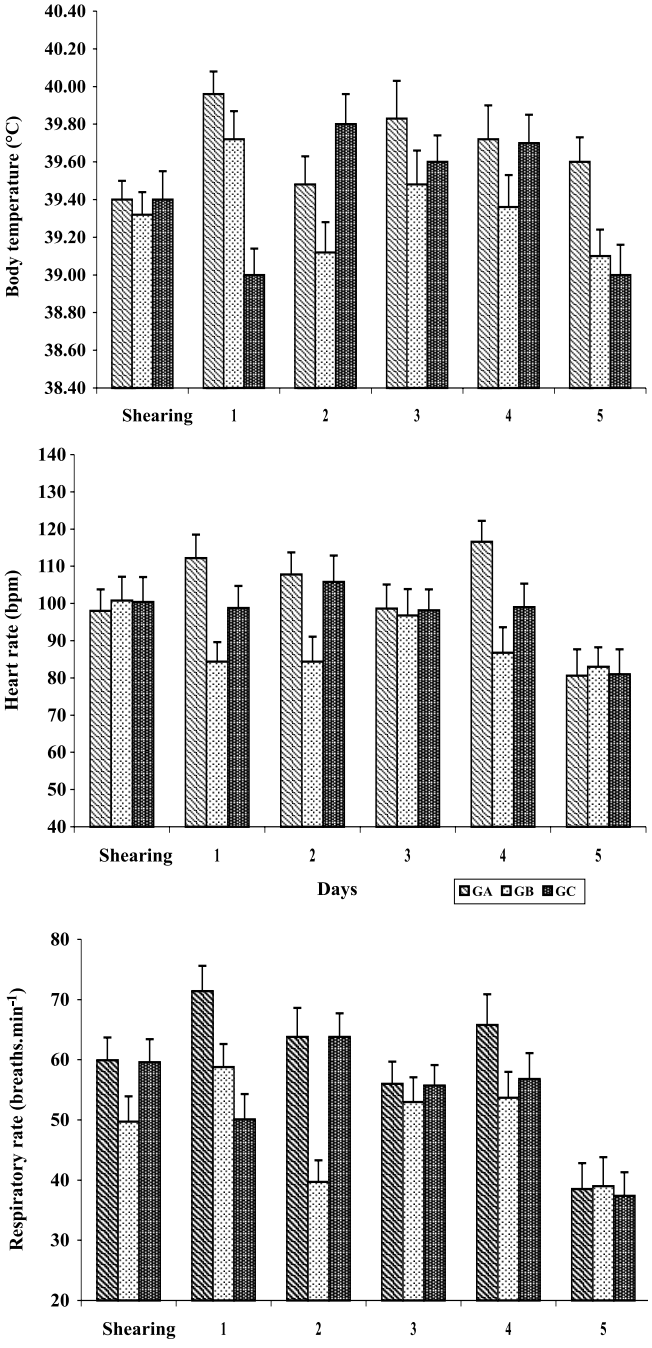

Fig. 1. Pattern (mean ± SD) of some physiological parameters (body temperature, heart rate and respiratory rate) observed in the different experimental conditions for groups A, B and C

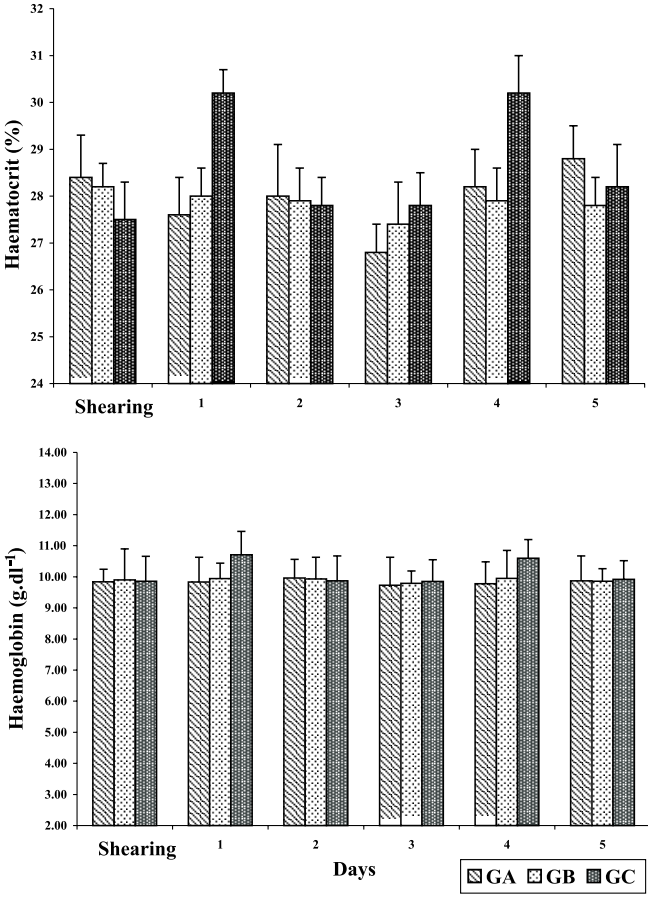

Fig. 2. Pattern (mean ± SD) of some haematological parameters (haematocrit and haemoglobin content) observed in the different experimental conditions for groups A, B and C

dispersal through conduction, radiation and evaporation should be aided by the absence of fleece: in fact, at an environmental temperature of 30 °C, a shorn sheep disperses 50 % more through evaporation than an unshorn sheep (2).

The ability to regulate temperature is an evolutionary adaptation that allows homeotherms to function in spite of variation in ambient temperature (1); this ability also allows temperature to be used as a signal to control physiological processes. Some authors have investigated the effects of different environmental conditions on physiological status of sheep (13). Many studies have been carried out in order to assess the influence of shearing and type of shelter on the homeostatic balance of sheep (3, 8—12): the authors show the important role of shelter's microclimate on the welfare of animals, underlining the beneficial effects which the removal of fleece has on metabolic levels of animals, with a subsequent optimization of productive performance.

The pattern of some physiological and haematological parameters (body temperature, heart rate, respiratory rate, haematocrit and haemoglobin content) have been investigated to evaluate the role played by the fleece and the influence of shelter on thermic homeostasis maintenance in Comisana ewes bred in Sicily.

MATERIALS AND METHODS

Thirty Comisana ewes, one year old, clinically healthy and well-fed yearlings, were used. They were divided into three groups, each consisting of ten subjects, housed in three different shelters: group A was kept in an outdoor pen, group B was kept in a pen equipped with a shady net (80 % of shading) and group C was kept in an outdoor sheepfold with an attached paddock. As concerning feeding conditions, groups A and B were fed daily on hay (2 kg), wheat straw (1 kg), wheat concentrate (0.5 kg) and water *ad libitum*; the remaining ewes grazed on a wheat stubble pasture for 6 hours per day,

and were sheltered during the midday warmer hours. On each subject through simultaneous monitoring equipment (Schiller Argus Tm 7) body temperature, heart rate and respiratory rate were measured.

Blood samples were collected by means of jugular venipuncture, to assess haematocrit (by a microhaematocrit method) and haemoglobin (by U. V. spectrophotometry). The studied parameters were assessed for each subject, every day at 08.00, with the following experimental conditions: before shearing, 1st, 2nd, 3rd, 4th and 5th day after shearing.

The statistical elaboration of the data for each parameter was based on the average values obtained at each day of test. The paired and unpaired *t*-test within each group and between groups respectively was applied to point out statistical significances among the average values of the studied parameters.

RESULTS

Figures 1 and 2 show the pattern (mean ± SD) of some physiological (body temperature, heart rate and respiratory rate) and haematological parameters (haematocrit and haemoglobin content) observed on the different experimental conditions for groups A, B and C.

Environmental temperature during the experimental period was unchanged (min.: 19 °C, max.: 25 °C). Data elaboration pointed out statistically significant differences for all the parameters studied in each group.

Body temperature showed a statistically significant increase before shearing in sheltered ewes (groups A and B) ($P < 0.0001$; $P < 0.001$) and a statistically significant decrease ($P < 0.01$) in grazed subjects (group C), compared to immediately after shearing. Body temperature was higher ($P < 0.01$) in group A compared to groups B and C 5 days after shearing. Respiratory rate showed a statistically significant increase one day after shearing in group A ($P < 0.01$) and group B ($P < 0.03$) and a statistically significant decrease in group C ($P < 0.01$) compared to before shearing.

Heart rate showed an increase one day after shearing in group A compared to groups B and C ($P < 0.001$). Both haematocrit and haemoglobin content showed higher values one day after shearing in grazed sheep (group C) compared to groups A and B ($P < 0.01$).

DISCUSSION

From the analysis of the obtained results, it was observed that different environmental conditions influenced the pattern of the studied parameters in each group. Before shearing all the subjects were within the thermoneutral zone (–4 °C, +28 °C) and heat production and heat dispersal mechanisms were equivalent. After shearing animals activated quickly heat production mechanisms for the raise of lower critical temperature (LCT).

The statistically significant decrease of body temperature in group C before shearing compared to immediately after shearing would be explained by wind cooling action, which could determine a remarkable convection heat loss higher than heat production in the grazing subjects. Five days after shearing this parameter showed higher values in group A ($P < 0.01$) compared to groups B and C: natural shelter in the pasture and shading net formed a more thermic comfortable microenvironment compared to group A, which were exposed to direct effect of solar radiation.

Respiratory rate showed a pattern similar to body temperature: it showed a statistically significant increase one day after shearing in group A ($P < 0.01$) and group B ($P < 0.03$) and a statistically significant decrease in group C ($P < 0.01$) compared to before shearing. Thermic polypnea is functional to disperse through evaporation heat stored in sheltered groups as a result of a rise in body temperature. Vice versa, a body temperature decrease in a grazing subject ($P < 0.01$) is the result of a respiratory volume decrease to a minimum value able to provide gas exchanges (7).

In sheep, panting is the major evaporatory heat loss mechanism and respiratory frequencies tend to follow closely the heat loss by evaporation (5). In non-sheltered sheep the respiratory rate can be 56 % higher than in sheltered sheep due to the direct effect of solar radiation (15).

Heart rate showed an increase one day after shearing in group A compared to groups B and C ($P < 0.001$), due to stress caused by the direct effect of solar radiation. Heat stress induces haemodynamic adaptations: the organism produces a higher quantity of heat dispersed by an enlarged peripheral vascular system.

Both haematocrit and haemoglobin content showed higher values one day after shearing in grazed sheep (group C) compared to groups A and B ($P < 0.01$), that would be due to a lower water availability during pasture compared to sheltered animals which have water *ad libitum*.

Obtained data show that different micro-environmental conditions, such as sheltering, can highly influence thermoregulatory mechanisms resulted from shearing and participate in the productive performance and welfare of sheep (15).

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COMPARISON OF THE EFFECTS OF IVERMECTIN ADMINISTRATION THROUGH THREE DIFFERENT ROUTES ON THE BLOOD PROFILES OF RATS

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ABSTRACT

Twenty adult rats were used in this study to compare the effects of administering ivermectin through three different routes on some haematological as well as serum biochemical parameters. The animals were divided into four groups of five animals each. Group A animals served as controls, hence did not receive any medicament while groups B, C and D animals on the other hand were administered with ivermectin through the subcutaneous (s/c), intramuscular (i/m) and intraperitoneal (i/p) routes respectively. The haematological parameters such as packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (HB), white blood cell count (WBC) and its differential, and erythrocytes indices were used to determine the effects of this drug on the rats. Biochemical parameters such as total protein, albumin, globulin, ALT, AST, ALP, BUN, creatinine, Na^+, K^+, Cl^-, Ca^{2+}, PO_4^{2-} were also used to assess the effects of the different routes of ivermectin administration on the blood profiles of rats.

The results of this study showed that most of these parameters were affected in about the same way for all the routes, signifying that there was no difference in the way the drug affects the blood profiles of rats irrespective of the routes of administration employed for drug delivery. The study also showed that ivermectin could be administered through any of these routes without any subsequent toxic side effects.

Key words: haematology; ivermectin; rats; serum biochemistry

INTRODUCTION

In the last decade Mectizan® (ivermectin), a novel anti-endectocide has displayed activity against a number of economically important arthropods such as ticks *Boophilus* spp. and *Amblyoma*, gastrointestinal nematodes like *Ascaris suum*, *Oesophagostomum* spp. – Gonzales *et al.* (15). There are a number of naturally occurring ivermectins arising as fermentation products of the Actinomycetes. The product of interest is 22,23, dihydroivermectin B1 – Prichard (25). This compound is active against developing larvae and the adult stage of important nematodes including those showing resistance to existing anthelmintic families. Ivermectin is a mixture of avermectin B1a and B1b. It is derived from soil actinomycete *Streptomyces avermitilis* – Brander *et al.* (5), Katzung (19). Ivermectin is formed by selective catalytic hydrogenation of avermectin – Ashraf *et al.* (3).

It was observed that a single subcutaneous injection of ivermectin could be detected in the blood as early as two hours post treatment – Marti (22). It has also been reported that

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with oral dosing the drug would attain two peak concentrations sixteen hours post treatment in the abomasal fluid using sheep as experimental models. The presence of this drug in higher concentrations in duodenal and iliac fluid indicates that this is the major route for the excretion of the absorbed drug — Marti (22).

Ivermectin is active against all stages of every major parasitic nematode including canine heartworm larva – Ottesen (24). It is effective against both internal and external parasites in different species of animals – Ottesen (24). It appears to paralyze nematode and arthropods (which may lead to death) by intensifying gamma aminobutyric acid (GABA) mediated transmission of signals in peripheral nerves – Ashraf *et al.* (3), Prichard (25), Katzung (19).

The intravenous injection into horses of a cattle formulation of ivermectin contrary to the recommended usage may cause immediate collapse with coma and periodic nystagmus. Intravenous injection of ivermectin is attended by a variety of untoward sequels such as ventral midline oedema caused by a reaction to dead microfilariae, limb oedema, eyelid oedema, fever, dyspnoea, disorientation, colic and sudden death. Transient swelling at the injection site is common – Blood *et al.* (4).

In clinical trials, Beagles and other dogs have shown adverse effects (mydriasis, tremor and ataxia) to single oral doses ranging from 2500 to 10000 $\mu g.kg^{-1}$ with death occurring at doses in excess of 9400 $\mu g.kg^{-1}$. The lethal dose (LD50) for Beagles is 80000 $\mu g.kg^{-1}$ – Burnham (6). It has been reported that clinical signs of ivermectin toxicosis in vertebrates are related to diffuse cerebral and cerebellar dysfunction which was true in two collies – Datry (10), Hovette (16).

Ivermectin is often used in the treatment of both external and internal parasites of dogs and other animals – Gann *et al.* (14), Okewole (23), Alayande *et al.* (1). A clinical worker inadvertently administered ivermectin intramuscularly to an Alsatian dog for a repeat treatment for helminth infection. The aftermath was an anaphylactic reaction. He was of the opinion that this reaction was due to the fact that he administered the drug through the wrong route in contrast to manufacturer's choice of subcutaneous route of drug administration. This study is therefore set to look at the effects of this agent on the blood parameters of rats using three different routes of administration (subcutaneous, intramuscular and intraperitoneal). This way the safest route of drug delivery for this agent would be determined.

MATERIALS AND METHODS

Animals and Experimental Designs

The animals used in this study were adult rats of the Sprague Dawley strain bred and maintained at the Animal House of the Faculty of Veterinary Medicine, University of Ibadan. They were kept in rat cages and fed rat cubes (Ladokun and Sons Livestock Feeds, Nigeria Ltd.) and allowed free access to clean fresh water *ad libitum*. They were divided into four groups of five animals per group. While the first group served as control the remaining three groups corresponded to the three different routes (subcutaneous, intramuscular and intraperitoneal) respectively. At the start of the experiment all the animals were weighed and recorded appropriately and the dose given was 0.2 $mg.kg^{-1}$ body weight. The effects of the drug on the animals were observed for three hours before the animals were killed and blood samples collected from them.

Technique for Obtaining Blood and Serum Samples

Blood was collected by cardiac puncture from diethyl ether anaesthetized rats into heparinised bottles for haematological studies. Blood sample was also collected into clean non-heparinised bottle and allowed to clot. The serum was separated from the clot and centrifuged (gravity) according to groups into clean bottles for biochemical analysis.

Determination of Haematological Parameters

Determination of haemoglobin concentration is as described by Jain (17) using the cyanomethaemoglobin method. Packed cell volume (PCV) was done by the conventional method of filling the capillary tubes with blood as described by Schalm *et al.* (26). Erythrocyte count was determined by the haemocytometer method as described by Coles (9). Total leukocytes and leukocyte differential counts were also determined. Erythrocytes indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined from values obtained from RBC count, haemoglobin concentration and PCV values.

Determination of Serum Biochemical Parameters

Total protein was measured using a biuret reaction while albumin was measured by colorimetric estimation using the Sigma Diagnostics albumin reagent (Sigma Diagnostic, U. K.), which contained bromocresol green (BCG). Globulin was obtained from the difference between total protein and albumin. Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were determined using a photoelectric colorimeter (Gallenkamp and Sons Ltd.; England) as described by Toro and Ackerman (28), Duncan *et al.* (12).

The determination of sodium and potassium ions in the sera was done using a flame photometer (Corning model 400, Corning Scientific Ltd; England) and the serum calcium level was measured by the cresolphthalein complexone technique by Toro and Ackerman (28). Serum phosphate level was determined using a photoelectric colorimeter (Gallenkamp and Sons Ltd.; England) as described by Bush (7). Serum urea and creatinine levels were determined using a photoelectric colorimeter (Gallenkamp and Sons Ltd.; England) as described by Toro and Ackerman (28) and Coles (9). The serum chloride level was estimated by the mercury titration method – Duncan *et al.* (12) and the serum bicarbonate level by back titration with sodium hydroxide – Toro and Ackerman (28).

Statistical Analysis

Results were subjected to ANOVA, DMRT and the Student's *t*-test and were considered significant at $P<0.05$ – Essex-Sorlle (13).

Table 1. Effects of the three routes on some haematological parameters of rats (n = 5)

PARAMETERS	A (Control)	B (s/c route)	C (i/m route)	D (i/p route)
PCV (%)	37.0 ± 2.0	43 ± 1.4[a]	44.0 ± 3.2[a]	43.0 ± 2.1[a]
Hb ($g.dl^{-1}$)	11.4 ± 0.5	14 ± 2.5[b]	15.0 ± 1.1[b]	14.0 ± 1.5[b]
RBC ($10^6.\mu l^{-1}$)	6.0 ± 0.3	7.0 ± 1.2[c]	7.0 ± 1.6[c]	7.0 ± 1.3[c]
MCV (fl)	60.8 ± 3.7	61.4 ± 2.1	62.9 ± 2.4	61.4 ± 2.6
MCHC (%)	31.2 ± 2.4	32.6 ± 0.5	34.9 ± 0.4	32.6 ± 0.9
MCH (pg)	19.0 ± 1.6	20.0 ± 0.6	20.0 ± 1.4	20.0 ± 0.6
WBC ($10^3.\mu l^{-1}$)	4.9 ± 0.4	4.0 ± 0.5	5.3 ± 0.3	8.8 ± 0.1[d]
Lymphocytes ($10^3.\mu l^{-1}$)	2.5 ± 0.8	2.5 ± 1.7	2.5 ± 1.4	4.2 ± 0.5[e]
Neutrophils ($10^3.\mu l^{-1}$)	2.0 ± 0.9	1.0 ± 1.5	2.4 ± 1.9	4.1 ± 0.9[f]
Monocytes ($10^3.\mu l^{-1}$)	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Eosinophils ($10^3.\mu l^{-1}$)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1

Note: Mean ± S.D. Superscripted items indicate significant values

Table 2. Effects of the three different routes on the serum chemistry of rats (n = 5)

PARAMETERS	A (Control)	B (s/c route)	C (i/m route)	D (i/p route)
Total protein ($g.l^{-1}$)	74.3 ± 2.1	60.0 ± 1.4[a]	65.0 ± 1.3[a]	60.0 ± 1.2[a]
Albumin ($g.l^{-1}$)	26.7 ± 1.5	27.0 ± 0.5	30.0 ± 1.1[bi]	24.0 ± 0.5[bi]
Globulin ($g.l^{-1}$)	47.6 ± 0.3	33.0 ± 0.2[c]	35.0 ± 0.6[ci]	36.0 ± 0.3[ci]
ALP ($U.l^{-1}$)	181.4 ± 2.6	86.6 ± 2.1[d]	83.5 ± 2.2[di]	86.8 ± 2.6[d]
ALT ($U.l^{-1}$)	51.5 ± 1.6	43.0 ± 0.5[e]	47.0 ± 0.4[ei]	45.0 ± 0.9[ei]
AST ($U.l^{-1}$)	97.5 ± 2.5	73.0 ± 0.6[f]	61.0 ± 1.4[fi]	72.3 ± 0.6[f]
BUN ($mmol.l^{-1}$)	11.0 ± 0.4	8.2 ± 1.5[g]	8.6 ± 1.3[g]	8.9 ± 1.1[g]
Creatinine ($\mu mol.l^{-1}$)	53.2 ± 0.8	123.8 ± 0.7[h]	114.9 ± 0.4[hi]	114.9 ± 0.5[hi]
Na^+ ($mmol.l^{-1}$)	131.2 ± 0.8	147.0 ± 1.5[j]	46.0 ± 1.2[ji]	149.0 ± 1.9[ji]
K^+ ($mmol.l^{-1}$)	7.1 ± 0.2	6.3 ± 0.1[k]	16.0 ± 0.1[k]	6.4 ± 0.1[k]
Cl^- ($mmol.l^{-1}$)	99.7 ± 0.4	105.0 ± 2.3	106.0 ± 2.2	103.0 ± 2.1
Ca_2^+ ($mmol.l^{-1}$)	8.2 ± 0.1	12.3 ± 0.4[m]	12.1 ± 0.3[m]	12.0 ± 0.2[m]
HCO_3^- ($mmol.l^{-1}$)	17.2 ± 0.1	20.0 ± 1.2[n]	23.0 ± 1.1[ni]	23.0 ± 1.5[ni]
PO_4^{2-} ($mmol.l^{-1}$)	4.8 ± 0.3	1.9 ± 0.5[o]	2.0 ± 0.3[o]	1.9 ± 0.8[o]

Note: Mean ± S.D.
Superscripted items indicate significant values between the control and the routes of administration.
Note that superscript i indicates significant difference between the subcutaneous route and other routes of drug administration at $P < 0.05$

RESULTS

Haematology

The erythrocytic and leukocytic values of the twenty rats studied are presented in Table 1. The results showed that PCV, Hb concentration and RBC levels of the animals in groups B (s/c route), C (i/m route) and D (i/p route) experienced a significant increase ($P<0.05$) over the control experiment. It was only the animals in group D that had a significant increase ($P<0.05$) in the levels of WBC, lymphocytes and neutrophils when compared with the controls.

Serum Biochemistry

The mean serum biochemical values of this study are presented in Table 2. The total protein, globulin, ALP, ALT, AST, BUN, K^+, Ca^{2+}, PO_4^{2-} of groups B, C and D animals all experienced a significant decrease ($P<0.05$) in their levels when compared with those of the control experiment. On the other hand, there was a significant increase ($P<0.05$) in the levels of creatinine, Cl^- and HCO_3^- of animals in groups B, C and D relative to the control experiment. For albumin, it was the animals in groups C and D that had a significant increase ($P<0.05$) in the level of this parameter when compared with those of the experimental group. It is to be noted however, that the following parameters experienced a significant difference ($P<0.05$) between the animals that received the drug through the subcutaneous route (group B) and other routes: albumin, globulin, ALP, ALT, AST, creatinine, Na^+ and HCO_3^-.

DISCUSSION

The haematological values recorded in this study showed that there was no difference in the effects of this drug on the blood profiles of rats irrespective of the route of administration employing in drug delivery. The subcutaneous route of administration is the manufacturer's preferred mode of drug delivery for this agent – Okewole (23). In this study it is clear that packed cell volume (PCV), haemoglobin concentration (Hb) and red

blood cell (RBC) count for all the experimental animals all experienced a significant increase over the control.

It is also interesting to note that there is no significant difference in the levels of these parameters when the routes of administration were considered. It just showed that any of the routes is safe for drug delivery. The significant increase noted for these parameters with the use of this drug through the three routes is particularly interesting. Since ivermectin belongs to the family of 16-membered macrocyclic lactones – Churchill (8), Prichard (25) – there may be the need to explore its relationship with erythropoiesis. In fact, it is said that some drugs can cause red cell increase due to the increased stimulation of the marrow by erythropoietin – Macfarlane (21).

The apparent increase in the haemogram of rats due to this drug may also be as a result of fluid shift from intravascular to the extravascular space due to increased vascular permeability – Straus (27). It may then be a case of relative polycythaemia. It was the group that received the drug through the intraperitoneal route that experienced significant increase in the levels of white blood cell (WBC) count, lymphocytes and neutrophils when compared with the control. All the same there was no significant difference between this route and other route with respect to these parameters.

With respect to the effects of the drug on plasma protein, it was discovered that there was a significant decrease in the levels of the total protein and globulin for all the experimental animals relative to the control. There was, however, a significant increase in the level of albumin in the groups administered with this drug through the intramuscular and intraperitoneal routes. It is said that alteration in the plasma total protein is most often due to the decrease in the quantity of albumin which may be accompanied by a relative hyperglobulinaemia – Coles (9). This is in contrast to the result of this study.

It is not easy to ascertain the reason for decreased total protein and globulin levels in this study. A study showed that administration of thyroxine in high doses for a sustained period of time led to accelerate protein catabolism and results in a negative nitrogen balance accompanied by diuresis – Dickson (11). Other causes of total protein depletion include age, nutrition, stress of extreme temperature and fluid loss by intestinal haemorrhages or exsudations have been identified to reduce total protein level – Allison (2), Kaneko (18), Coles (9).

Measurement of certain enzymes is the most commonly used screen for hepatic disease. Hepatocellular damage results in release of cellular enzymes with subsequent increases in serum levels. Increased liver enzymes in serum indicate altered hepatocyte permeability and or necrosis (hepatocellular enzymes) or cholestatic disease – Coles (9), Duncan *et al.* (12), Knoll (20).

In this study, there was a significant decrease in these enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). It thus showed that administering the drug through any of the route will not result in any damage to the liver. In this study too, there was a significant decrease in the level of blood urea nitrogen (BUN) but a significant increase in the level of creatinine.

Blood urea nitrogen and creatinine are the substances in the blood most often used to assess renal function – Duncan *et al.* (12), Knoll (20). Increases in BUN are most often associated with renal disease. High-protein diets and increased protein catabolism from fever or tissue necrosis can cause mild increases in BUN – Knoll (20).

Since there is a decrease in the level of this parameter, it also shows that the drug is safe for use through any of the routes of administration employed in this study. The increased noted for creatinine in this study could not be ascertained.

While plasma electrolytes such as sodium, potassium, calcium and phosphate experienced significant decrease in their levels, the reverse is the case for chloride and bicarbonate. The increase noted for chloride may be because macrocyclic lactones act by binding to a glutamate-gated chloride channel receptor in nematode and arthropod nerve cells. This causes the channel to open, allowing an influx of chloride ion that leads to flaccid paralysis – Prichard (25), Katzung (19).

It is therefore safe to conclude that the cause of the anaphylactic reaction experienced by the dog of the colleague is not necessarily due to the fact that the drug was administered through the intramuscular route but may be due to reaction to dead microfilariae especially since the drug was being administered for the second time in two weeks – Blood *et al.* (4). This study also showed that ivermectin is a very safe drug for use in controlling helminth infections in livestock animals.

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IMMUNOHISTOCHEMISTRY AND CANINE SKIN AND MAMMARY GLAND TUMOURS

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ABSTRACT

This study was focused on the use of selected antibodies in the differentiation of spontaneous canine skin and mammary gland tumours. The collective immunohistochemicall expression of 8.00 (low molecular weight – LMW) and 8.60 (high molecular weight – HMW) anticytokeratines peptide, desmin, vimentin, melanoma HMB 45 antibody, p53 tumours suppressor protein (DO-1), CD3 antiserum to human T-cells, S-100 protein, lysozyme, alpha-1 antitrypsin and proliferation cell nuclear antigen (PCNA) were analysed on formalin-fixed, paraffin-embedded and frozen tissue sections. The detection of vimentin, HMW cytokeratin, p53, CD3 cells, lysozyme and PCNA were the most useful. There was a less consistent and lighter positive response to desmin in all tumours. The reaction response to LMW cytokeratin was very low. The use of S-100 protein HMB 45, alpha-1 antitrypsin antibody as markers in canine skin and mammary gland tumours is not recommended. The findings in this study indicate that immunohistochemistry applied to tumours of unknown cellular origin is of a great potentional diagnostic benefit in veterinary pathology. But the usefulness of the immunohistochemical detection of tumours by different types of markers will be only shown when these stains have been standardised among many laboratories and a large body of information concerning these staining patterns has become available.

Key words: canine mammary gland and skin tumours; immunohistochemistry

INTRODUCTION

Cancer is a common diagnosis and frequent cause of death or euthanasia all over the world. In recent years considerable interest has been focused on the occurrence of tumours of the skin and mammary glands in dogs. They present a diagnostic challenge to the pathologist and often pose problems of differential diagnosis when examined by light microscopy. However, immunohistochemistry has provided a technique with which polyclonal and/or monoclonal antibodies can be used to gain more diagnostic information about a variety of neoplasms (13). Over the past few years, a number of tumour markers for the differentiation of tumours have been described (9, 17).

In our study a panel of selected commercially available antibodies was used for the examination of spontaneous canine skin and mammary gland tumours of various breeds and age.

MATERIAL AND METHODS

Dogs of different breeds and age with suspected neoplastic changes of the skin and mammary glands were examined. For histological examination 170 samples were taken from changed areas. They were:

1. fixed in 10 % neutral formalin, embedded in paraffin and stained with haematoxylin and eosin;
2. collected in phosphate buffered saline (PBS) and cut on Cryocut E (Reichert Jung).

6 μm thickness frozen tissue sections were mounted on glass slides, dried at room temperature, fixed in cold acetone

Table 1. Details of primary antibodies used

Specific antibody used	Pretreatment	Type	Dilution	Source
LMW Cytokeratine	Digested	Monoclona antimouse	1:50	Biogenex
HMW Cytokeratine	Digested	Monoclonal antimouse	1:50	Biogenex
Desmine	Undigested	Polyclonal antirabbit	1:8	Biogenex
Vimentine	Undigested	Polyclonal antirabbit	1:10	Biogenex
HMB 45	Undigested	Monoclonal antimouse	1:10	Biogenex
DO-1 (p 53)	Undigested	Monoclonal antimouse	1:2000	Biotechnology
CD3	Digested	Polyclonal antirabbit	1:300	Dakopatts
S-100	Undigested	Polyclonal antirabbit	1:40	Biogenex
Lysozyme (Muramidase)	Digested	Polyclonal antirabbit	1:300	Dakopatts
Alpha-1-Antitrypsin	Digested	Pyclonal antirabbit	1:100	Dakopatts
PCNA	Digested	Monoclonal antimouse	1:44	Dakopatts

for two minutes and stored in a refrigerator. Frozen sections were used for HMB 45 and DO-1 antibodies detection.

The reaction response to immunohistochemical markers was achieved by means of the Biotin-Streptavidin amplified peroxidase detection (B-SA) system (Biogenex, Sam Ramon, CA, USA). After using the DO-1 antibody against p53, sections were treated in a microwave oven in 0.01 mol citrate buffer (pH 6.0) for fifteen minutes. The immunological reactions were revealed by diaminobenzidine (DAB) substance.

The examination of proliferating cell nuclear antigen (PCNA) was carried out according to a specially included protocol (DAKO, A/S, Glostrup, Denmark) with:

1. Monoclonal mouse anti-PCNA (Clone PC10) antibody (DAKO, A/S, Glostrup, Denmark) and
2. Animal Research Kit (ARK), which contains all components (peroxidase block, streptavidin HRP, blocking reagent, biotinylation reagent and DAB tablets), needed for examination.

The antibodies used are summarised in Table 1.

All samples were evaluated microscopically to determine the presence and cellular location of positive staining. Staining was evaluated for the following characteristics: 1. staining intensity – absent, light, or dark brown, 2. cellular location of the staining – cytoplasmic or nuclear, 3. tissue staining pattern – focal, multifocal, or diffuse.

Negative controls were obtained omitting the primary antibodies.

RESULTS

In the histological study there was a higher prevalence of malignant tumours (52.35 %) than benign (47.64 %). Squamous cell carcinoma from skin tumours and the complex of benign mixed tumours occurred most frequently as well as adenocarcinomas from mammary gland tumours (Table 2).

The results of immunohistochemical staining are summarised in Table 3.

DISCUSSION

Canine mammary gland and skin tumours were stained with different types of immunohistochemical markers to determine the availability for their use in routine diagnostic work.

About 20 different types of cytokeratines have been described, which is probably why there are several monoclonal antibodies against keratines of different molecular weights. They can recognise epithelial cells in formaline-fixed specimens. It has been observed that so-called simple as well as glandular epithelia express keratines of a lower molecular weight, while stratified epithelia express high molecular weight keratines. Neoplastic epithelial cells in canine mammary gland and skin tumours expressed mainly keratines of stratified epithelia rather than those of simple epithelia (16). Similarly, our results indicate a very good expression of HMW cytok-

Table 2. Observed skin and mammary gland tumour types

The incidence of tumours from 170 examined (%)			
Skin (67.50 %)		**Mammary gland (32.94 %)**	
	benign		**benign**
10.59	*adenoma sebaceum*	11.16	benign mixed tumours/fibroadenomas/complex adenomas
7.65	*papilloma*	1.18	*lipoma*
2.94	*histiocytoma*	0.59	*myxofibroma*
6.47	*fibroma*		
2.35	*haemangiopericytoma*		
4.11	*epulis fibromatosa*		
2.94	*lipoma*		
0.59	*trichoepitelioma*		
	malignant		**malignant**
13.53	*squamous cell carcinoma*	4.12	*adenocarcinoma cysticum*
10.58	*basal cell carcinoma*	3.53	*adenocarcinoma papilliferum*
3.53	*fibrosarcoma*	3.53	*carcinoma solidum*
2.94	*mastocytoma*	2.94	*adenocarcinoma tubulare*
2.35	*sebaceous gland adenocarcinoma*	2.94	*fibrosarcoma*
		2.94	*carcinoma medullare*
		0.59	*carcinoma scirrhoticum*

Table 3. Results of immunohistochemical staining

	HMWC	LMWC	Desmin	Vimentin	S-100	CD3	p53	HMB45	Lysozyme	Trypsin	PCNA
MAMMARY GLAND											
Adenocarcinoma	+	+	–	+	–	+	+	–	+	–	+
Fibrosarcoma	–	–	+	+	–	+	+	–	+	–	+
Fibroadenoma	+	–	+	+	–	–	+	–	–	–	+
SKIN											
Squamous cell carcinoma	+	–	–	+	–	+	+	–	–	–	–
Fibrosarcoma	–	–	+	+	–	+	+	–	+	–	–
Histiocytoma	–	–	–	+	–	+	–	–	+	–	–
Sebaceous gland adenoma	+	–	–	–	–	+	+	–	–	–	+
Fibroma	–	–	+	+	–	–	–	–	–	–	–

eratine in the chosen tumours (Fig. 1) in contrast to the expression of LMW cytokeratine, which was observed only in adenocarcinoma of mammary gland.

All tumours reacted to the vimentine polyclonal antibody in a diffuse and intense fashion. An immunoreaction response was observed in the majority of tumour cells (Fig. 2). This antibody showed the most marked reaction out of all tested antibodies and it is very useful for the immunohistochemistry of canine skin and mammary gland tumours.

The detection of desmine in formalin-fixed paraffin-embedded tissue was less consistent and lighter than that of other intermediate filaments. As with the others (4) we can recommend the demonstration of this intermediate filament in frozen sections because formaline fixation had a deleterious effect on the antigen response of desmin.

The tissue–specific distribution of intermediate filament proteins are retained in most neoplastic cells and their detection is used for distinguishing between epithelial and mesenchymal tumours (7, 5, 1). This study has demonstrated that commercially available monoclonal antibodies to intermediate filaments developed for use in the identification of human tumours recognise similar antigens in normal and well-differentiated neoplastic canine tissues. Since intermediate co-expression can

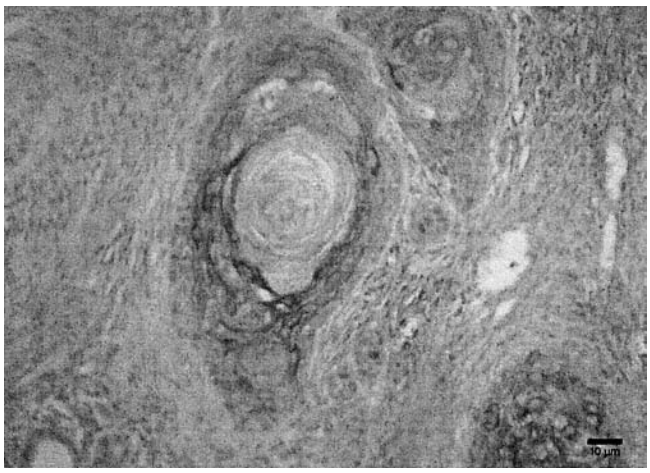

Fig. 1. Brown cytoplasmic focal expression of HMW (high molecular weight) cytokeratine in squamous cell carcinoma (Formalin-fixed, paraffin embedded tissue, B-SA system, 20 ×)

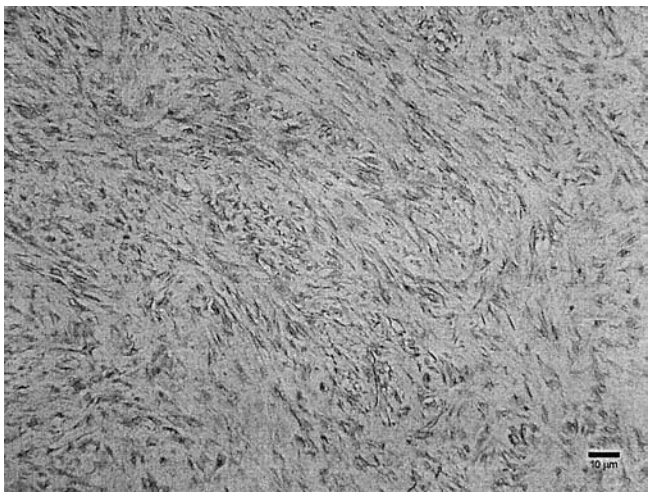

Fig. 2. Brown diffuse reaction of vimentine antibody in the majority of fibroma molle tumour cells (Formalin-fixed, paraffin embedded tissue, B-SA system, 20 ×)

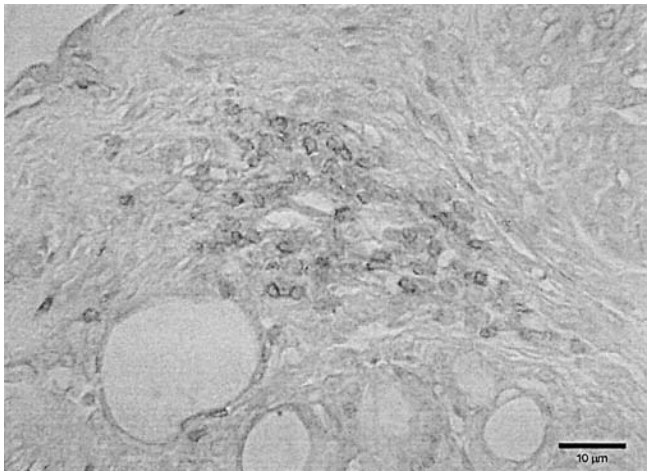

Fig. 3. Brown positive response to CD3 lymphocytes in mammary gland adenocarcinoma (Formalin-fixed, paraffin embedded tissue, B-SA system, 40 ×)

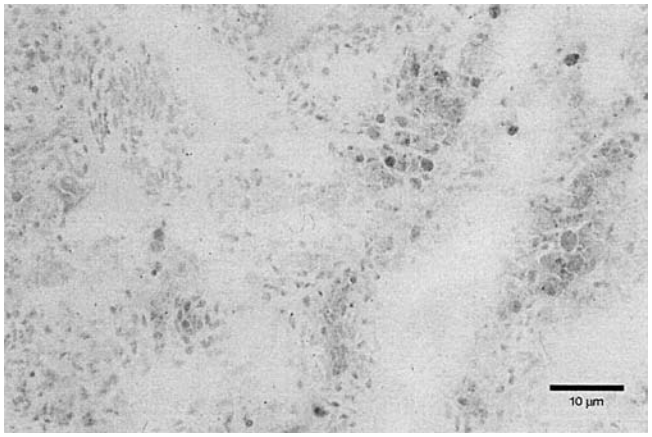

Fig. 4. Brown nuclear expression of p53 in adenocarcinoma of the mammary gland (Frozen section, fixed in aceton, B-SA system, 40 ×)

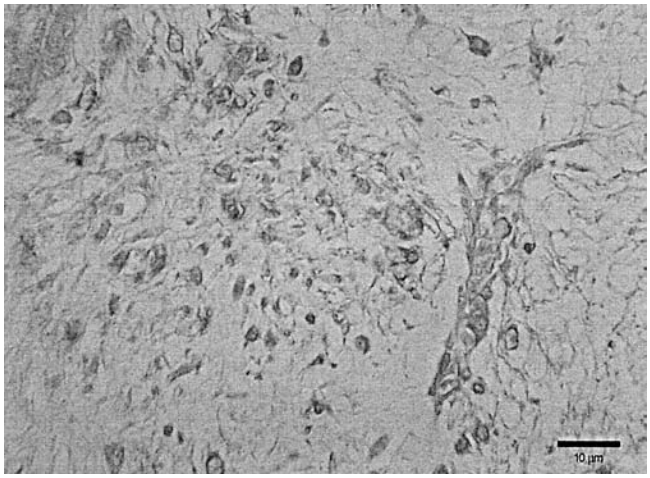

Fig. 5. Dark brown nuclear multifocal positive response to lysozyme in macrophages of peripheral inflammatory reaction in feline postvaccinated fibrosarcoma (Formalin-fixed, paraffin embedded tissue, B-SA system, 40 ×)

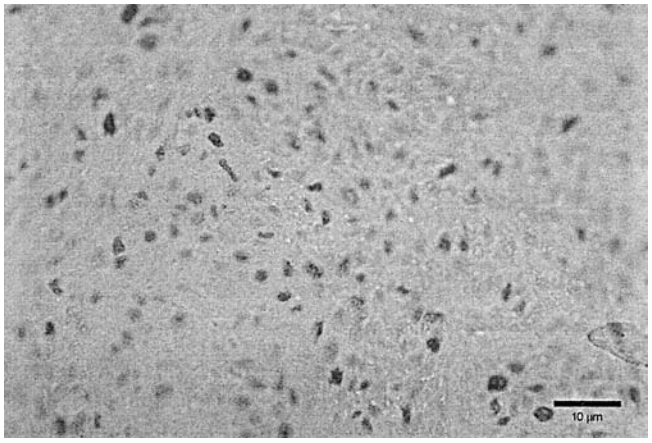

Fig. 6. Brown nuclear multifocal positive response to proliferating cell nuclear antigen in fibrosarcoma of the mammary gland (Formalin-fixed, paraffin embedded tissue, B-SA system, 40 ×)

occur in neoplasm, it is important to stain tumours with a series of antibodies to assist in obtaining an accurate diagnosis.

The positive response of CD3 lymphocytes was confirmed in adenocarcinoma and fibrosarcoma of mammary gland and in squamous cell carcinoma, histiocytoma (Fig. 3) and adenoma of the skin. Polyclonal anti-CD3 antibody has been successfully used in veterinary medicine in several times under different pathological processes and different animals (10, 18). Moreover, we have had very good experience with this antibody on formaline-fixed paraffin-embedded cutaneous lymphoma of dogs. CD3 lymphocytes were clearly visible and they showed a brown reaction in the cell membrane and cytoplasm (unpublished data).

The mononuclear cell infiltration into cancers is not always a measure of the host immune response but in experimental studies some authors have found a significant increase of B-lymphocytes in the progressive stage of tumours, whereas a significant increase of T-lymphocytes has been found infiltrating the regressive stage (19). In this way, the immunostaining of T-lymphocytes with the polyclonal anti-CD3 antibody in routine tissue sections facilitates the identification and quantification of T-lymphocytes, and anti-CD3 antibody could be useful in evaluating the regressive growth stage of the tumour.

The discovery of tumour suppressor genes, whose loss of its function may elicit tumorigenesis, has provided a new approach to understanding of tumour biology. One of the best-known tumour suppressor genes is the p53 gene (20). In fact, normal cells with the wild type p53 gene do not show p53 protein immunoreaction response, whereas tumours with a mutant form of p53 gene may express a high level of p53 protein.

The immunohistochemical demonstration of p 53 in neoplasms and its increased expression in malignant neoplasms provides additional information on the character of the growth that may prove useful in assessing tumour progression and hence prognosis in clinical cases (6). We observed the expression of p53 in all the examined tumours (Fig. 4) except skin histiocytoma and fibroma. The positive nuclear staining was observed as finally granular, with a well-distributed brown coloration evident throughout the nuclei of the affected cells. DO-1 monoclonal antibody seems to be the useful marker for routine confirmation of p53 in canine neoplastic tissue.

HMB 45 antibody was designed for the qualitative localisation of malignant melanoma in formaline-fixed paraffin-embedded or frozen tissue sections. There is a positive response to HMB 45 by melanoma and junctional nevus cells but not by normal melanocytes or intradermal nevus cells. There was also a successful use of this antibody in chronic dermatitis in dogs in our department. Their skin showed pigmented defects caused by a failure of transference to hyperplastic keratinocytes. They were free of melanin and the melanocytes were filled with melanin (unpublished data).

Alpha-1-antitrypsin is a 51kDa glycoprotein normally present in liver cells, histiocytes, monocytes and in a large variety of tumours of both epithelial and mesenchymal differentiation (1).

The S-100 protein is a group of low molecular weight (10—12 kD) calcium–binding proteins very evident in vertebrates (2). But its reaction response in normal canine tissues has not been thoroughly investigated (11). S-100 protein detection has been used in the diagnosis of benign sheath nerve tumours and melanomas but also in a variety of non-neural and non-melanotic tumours such as canine and feline malignant histiocytomas, human breast carcinomas and human haemangiopericytomas (15).

Our results did not show any positive reaction to HMB 45, S-100 protein and alpha-1 antitrypsin either in skin or mammary gland tumours.

The expression of lysozome shows the presence of monocytes, granulocytes and also histiocytic differentiation. In tissues of mesenchymal origin, lysozyme is considered a histiocytic marker both in humans and dogs (15). In our study there was a marked positive response to lysozyme in mammary gland fibrosarcoma, skin histiocytoma and feline postvaccinated fibrosarcoma. The macrophages of mild peripheral inflammatory reaction showed a multifocal dark brown reaction in the cytoplasm (Fig. 5).

The proliferating cell nuclear antigen (PCNA) has recently been used to identify replicating cells (3). It is one of the central molecules responsible for the decision of life and death of the cell (14). It is a delta accessory protein of DNA polymerase and is synthesised in the late G1 and S phase of cell cycle. PCNA therefore correlates with cell proliferate state. PCNA is present throughout the cell cycle (8) and has a triple function in the life and death of the cells. When not engaged in DNA replication, PCNA (most often under the control of p53) commits cell to cell cycle arrest and repair of DNA damage, or, when repair is not possible, the absence or low levels of functional PCNA may drive cells into apoptosis (14). The advent of commercially available antibodies of PCNA has allowed the immunohistochemical detection and evaluation of this protein (3). They have been successfully used as markers of tumour cell proliferation activity so that the rapidity of tumour growth and the prognosis of the cancer could be established. In our study the expression of PCNA was confirmed in sebaceous gland adenoma and in mammary gland tumours (Fig. 6).

The staining of canine skin and mammary gland neoplasms confirms the tissue-specificity of intermediate filament and the expression of other markers in these tumours and suggests it as a useful technique for the identification of these types of tumours in dogs.

It is important to guard against false negative staining, which may result from the effects of fixation and processing. This is apparent when normal tissues present within a neoplastic section fail to stain for the expected intermediate filaments. In addition, since keratines consist of twenty different proteins, some anti-keratin monoclonal

antibodies may not detect all keratines and, therefore may fail to detect an epithelial neoplasm.

Immunohistochemical staining of canine skin and mammary gland tissues with commercially available antibodies directed against human antigens such as anticytokeratine peptide 8.60 (high molecular weight – HMW), desmin, vimentin, p53 tumours suppressor protein (DO-1), CD3 antiserum to human T-cells, lysozyme and PCNA indicates that these antibodies can be used for a more accurate diagnosis and classification of canine mammary gland and skin tumours.

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THE INFLUENCE OF PARENTERAL ADMINISTRATION OF ZINC ON THE REPRODUCTIVE PERFORMANCE OF BOARS

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ABSTRACT

The preparation Zindep inj. a.u.v. (BIOTIKA, SR) was administered by single injection to seven boars in an experimental group on day zero in a dose recommended by the producer (10 mg of zinc per kg of live weight) by intramuscular application. The blood of boars was collected on the 21st and 71st days of the experiment. The quantitative and qualitative parameters of the boars' semen were also evaluated. Administration of Zindep inj. statistically significantly influenced some quantitative and qualitative parameters of boars' semen: sperm concentration, total number of spermatozoa per ejaculation, the motility of spermatozoa after collection, thee motility of spermatozoa in the insemination dose 24 and 48 hours after dilution and the total number of morphologically abnormal spermatozoa. Zindep inj. also significantly affected the percentage of the boars' fertility after the first insemination.

Key words: boar; quantitative and qualitative parameters; reproductive performance; semen; zinc

INTRODUCTION

Zinc is an essential element for living organisms of all species. It is an important component of metaloenzymes, such as alkaline phosphatase, glutamate-dehydrogenase, lactate-dehydrogenase, malate-hydrogenase, carboanhydrase, tryptophan desmolase (1).

Zinc directly affects the male and female reproductive organs through the pituitary gonadal axis. In males it controls the release of pituitary gonadotropins, androgene and testosterone (14). A deficiency of zinc stops the development of the testes and causes atrophy of the seminiferous epithelium. This results in the total impairment of spermatogenesis (3, 5) manifested by a lower fertilization capability of the semen (1). The motility and total concentration of sperms and percentage of surviving sperms decreases in zinc deficient breeding males (4) and as result of this fertility is also decreased (17).

Zinc deficiency has caused degenerative changes in the seminiferous epithelium and their depletion. The increased occurrence of malformed spermatids is indicated by an impaired course of spermatogenesis. Degeneration of Sertoli cells and Leydig cells has been observed (5). On the basis of the above mentioned data one can state that an adequate supply of zinc is one of the necessary preconditions for the optimum reproductive capability of breeding males.

The aim of our study was to evaluate the biological effectiveness of a zinc-based preparation, Zindep inj, with regard to the zinc and copper concentration in blood, the selected quantitative and qualitative parameters of semen and reproductive indicators of boars in stud.

MATERIAL AND METHODS

The investigations of the effectiveness of the preparation Zindep inj. were carried out with 13 boars. The conrol group consisted of six boars, 18.67 ± 0.92 months old and the experi-

mental group consisted of seven boars, 26.14 ± 4.78 months old. The average live weight in the control group was 246.7 ± 11.45 and in experimental group 280.9 ± 23.17. The boars were kept in the same housing, feeding and tending conditions. The clinical examination of all the animals was performed before the beginning of the experiment.

The zinc-based preparation Zindep inj. a.u.v. (Biotika, the Slovak Republic) was administered by a single injection intramuscularly (at a dose of 10 mg $Zn.kg^{-1}$ of body weight) into seven experimental boars at the beginning of July. The total dosage for boars of live weight 246.70 ± 11.45—280.9 ± 23.17 kg is 39.2—50.7 ml per animal. This dosage must be divided into 6.5—8.5 single injections with a maximum volume of 6 ml per one injection. The producer of the preparation reports a long-term duration effect and from this point of view it is not appropriate to carry out repeated administration three to four months prior to investigation (Biotika, the Slovak Republic).

The experiment was divided into three parts. A period before the experiment (January–June), a period during the experiment (Zindep inj. administration; July–September) and a period after the experiment (October–December).

Blood samples were taken from the *vena jugularis* and *sinus ophthalmicus* of the experimental and control boars during the experiment (July–September). The beginning and duration of the effect of the preparation in accordance with producer recommendation was taken note of at the collection of blood on day 0 (before the administration of Zindep inj.) and on the 21st and 71st days of the experiment. The determination of zinc and copper in the blood serum was carried out by the AAS (atomic absorbance spectrophotometry) method using the apparatus Perkin Elmer (5000). The values of zinc and copper in blood serum are expressed in $\mu mol.l^{-1}$ and those in the diet in $mg.kg^{-1}$ in DM.

The quantitative and qualitative parameters of the boars' semen were analysed between control and experimental group at the collection of semen during the whole period of the experiment (January–June; July–September; October–December). Semen volume, sperm concentration, the total number of spermatozoa per ejaculation, the motility of spermatozoa after collection, the motility of spermatozoa in the insemination dose after 24 hours and 48 hours after dilution were evaluated.

The semen was collected once to twice a week from all boars on the phantom manually, always between 6 a.m. and 9 a.m. The spermatic fraction was measured at the same time. The semen was collected and evaluated by the same staff. The volume of the fluid was measured in a graduated tube with accuracy in 0.5 cm^3. The semen collection frequency took note of a boar's age, temperament, health status, ejaculation quality at the last semen collection and also the demands of production insemination doses per sows.

The samples of seminal fluid taken from the control and experimental boars were used to estimate the activity of the sperm. The concentration of sperms in 1 mm^3 was determined by the haemocytometric method. Smears prepared for morphological analysis were stained according to Giemsa-Romanowski and evaluated microscopically with a magnification of 1500× under immersion. One hundred sperms were evaluated in each preparation (7).

The motility of sperms in fresh seminal fluid taken from the control and experimental boars were evaluated after its dilution with 3.1 % sodium citrate at a ratio of 1:1—5. The survival of sperms was analysed in insemination doses of boars at 24 hour intervals for 48 hours. The diluted semen was stored at 15 up 16 °C during the observation.

The total number of spermatozoa per ejaculation (TNS) of the experimental and control boars (volume of seminal fluid x concentration of sperms in 1 mm^3) was evaluated. The spermological data obtained were presented as means of the respective values.

The reproductive indicators of boars were observed during the time of farrowing sows (November to January), until the time of weaning pigs. We monitored the sows that were inseminated by the semen of boars during the experiment (July–September). We analysed the number of piglets born per litter, the number of piglets born alive per litter, the number of pigs weaned per litter and the percentage of the boar fertility after the first insemination. During the whole time of the experiment only artificial insemination with reinsemination was used. The detection of oestrus was performed on the basis of external signs oestrus, by compressively testing a sow and by using the boars'-locater.

The results of the analysis of zinc in blood serum are presented as mean values and standard deviations (± SD). GraphPad Prism 3.0 (1999) using Student's *t*-test was applied to compare the results (14). Fischer's analysis of variance (ANOVA) was used in to compare the results of the boars in the experimental group.

RESULTS AND DISCUSSION

Before the administration of the preparation, a marked hypozincaemia was registered in the blood serum of the experimental and control animals. The concentration of zinc in blood serum was (n = 6) on day zero 9.30 ± 0.36 $\mu mol.l^{-1}$ in the control group and in the experimental group (n = 7) 10.70 ± 0.71 $\mu mol.l^{-1}$. The difference between mean values of the control and the experimental group are not statistically significant ($P > 0.05$).

The concentration of zinc in blood serum of the boars on day 21 of the experiment was in the experimental group 14.41 ± 0.54 $mol.l^{-1}$ and in the control group 10.70 ± 0.28 $\mu mol.l^{-1}$. The difference between the serum levels of Zn in the experimental and control animals was significant until day 71 of the experiment ($P < 0.01^{**}$). The concentration of zinc in blood serum of boars in the experimental group was 12.45 ± 0.43 $\mu mol.l^{-1}$ and in the control group 9.30 ± 0.80 $\mu mol.l^{-1}$ on this day.

The administration of preparation Zindep inj. in a dose recommended by the producer 10 mg of zinc per kg of live weight by intramuscular application induced no local changes at the site of injection.

The difference in the age of boars and in the live weight of boars between the control and the experimental group was not statistically significant ($P > 0.05$).

Semen collection frequency before (January–June)

and after the experiment (October–December) between the control and the experimental group was not statistically significant (P>0.05). Semen collection frequency between the control and the experimental group during the experiment (Zindep inj.; July–September) was statistically significant (P<0.05*) (Tab. 1).

Table 1. Semen collection frequency (semen collection per month) in the period before the experiment (1—6), during the experiment (7—9: Zindep inj. administration) and after the experiment (10—12) between control and experimental groups

Calendar months	Control group	Experimental group	*t*-test
1—6	5.2 ± 0.13	4.95 ± 0.12	P > 0.05
7—9	5.3 ± 0.29	4.71 ± 0.21	P < 0.05
10—12	4.73 ± 0.44	5.29 ± 0.21	*P > 0.05

According to Cameron (2) semen collection three times per week, on Monday, Wednesday and Friday, resulted in a greater volume, concentration and total sperm per ejaculation than continued 24 or 48 hour collection intervals. The three times a week scheme may result in a greater output because the boars appeared to maintain a higher level of *libido*. High collection frequencies resulted in decreased sperm motility, concentration, total sperm and an increased percentage of abnormal sperm and sperm with damaged membranes. Osmotic resistance-test values were also decreased (16). The total number of spermatozoa per ejaculation in all boars is dependent above all on the length of sexual rest. The highest level was measured after an interval six to seven days between collections (6).

Semen volume between control and experimental group was statistically very significant during the whole time of the experiment (P<0.001***).

On the basis of the ANOVA test the semen volume in the period before the experiment (January–June) and during the experiment (July–September) (268.7 ± 6.54 vs. 290.5 ± 8.33), in the period during experiment and after the experiment (October–November) (290.5 ± 8.33 vs. 270.7 ± 6.56) and in the period before the experiment (January–June) and after the experiment (268.7 ± 6.54 vs. 270.7 ± 6.56) in the experimental group of boars was not statistically significant (P>0.05) (Tab. 2).

Semen collection frequency at the insemination station took note of age, temperament and health status and qualitative parameters of ejaculation at the last semen collection. Semen collection was performed according to the need of production insemination doses on the number inseminated sows. The semen collection between the control and experimental group was statistically significant during the experiment (July–September). The boars in the insemination station were not excessively sexually exploited during the whole period of the experiment. Semen volume depends on: age, nutrition, breed, individuality of boar, live weight, season, semen collection frequency, and the semen collection method. Semen volume quantity is an indicator of androgen activity, fixed genetically (9).

Table 2. Semen volume in the experimental group of boars in the period before (1—6) and during the experiment (7—9): Zindep inj. administration), in the period during (7—9) and after the experiment (10–12) and in the period before (1—6) and after the experiment (10—12)

Calendar months	Semen volume(ml)	Semen volume(ml)	ANOVA
1–6 vs. 7–9	268.7 ± 6.54	290.5 ± 8.33	P > 0.05
7–9 vs. 10–12	290.5 ± 8.33	270.7 ± 6.56	P > 0.05
1–6 vs. 10–12	268.7 ± 6.54	270.7 ± 6.56	P > 0.05

Sperm concentration in the period before the experiment (January–June) was not statistically significant (P>0.05). In the period during the experiment (July–September) and in the period after the experiment (October–December) we registered a statistically significant increase in the sperm concentration of boars in the experimental group in comparison with the control (P<0.001***) (Tab. 3).

Table 3. Sperm concentration (in 1 mm^3) in the period before the experiment (1—6), during the experiment (7—9: Zindep inj. administration) and after the experiment (10—12) between the control and experimental groups

Calendar months	Control group (×10^3)	Experimental group (×10^3)	t-test
1—6	251.9 ± 1.34	251.2 ± 1.34	P > 0.05
7—9	239.5 ± 0.29	266.5 ± 2.73	P < 0.001***
10—12	234.2 ± 0.96	247.6 ± 1.45	P < 0.001***

Oral supplying of zinc in the diet for boars increased semen volume (nearly twice) and also increased the total number of spermatozoa per ejaculation (TNS). The vitality and resistance of boar sperms after the addition of zinc was 2.5 times higher (12). Administration of Zindep inj. increased sperm concentration in the ejaculation of boars in the experimental group and remained significant until the end of experiment.

The total number of spermatozoa per ejaculation between the control and experimental groups was statistically very significant during the whole time of the experiment (P<0.001***).

Table 4. The total number of spermatozoa (TNS) per ejaculation in the experimental group of boars in the period before (1—6) and during the experiment (7—9): Zindep inj. administration), in the period during (7—9) and after the experiment (10—12) and in the period before (1—6) and after the experiment (10—12)

Calendar months	TNS (mld)	TNS (mld)	ANOVA
1–6 vs. 7–9	67.2	77.4	P < 0.05
7–9 vs. 10–12	77.4	67.0	*P < 0.01
1–6 vs. 10–12	67.2	67.0	**P > 0.05

An increase in the total number of spermatozoa per ejaculation according to the ANOVA test in the experi-

mental group of boars in the period during the experiment (July–September) in comparison with the period before the experiment (January–June) was statistically significant ($P<0.05^{*}$). A decrease in TNS in the period after the experiment (October–December) in comparison with the period during the experiment is statistically significant ($P<0.01^{**}$). A decrease in TNS in the period after the experiment (October–December) in comparison with the period before the experiment (January–June) is not statistically significant ($P>0.05$) (Tab. 4).

The motility of spermatozoa after collection in the period before the experiment (January–June) between the control and experimental groups was not statistically significant ($P>0.05$). An increase in the motility of sperm after administration of Zindep inj. (July–September) in the experimental group of boars was statistically very significant until the end of experiment (October–December) ($P<0.001^{***}$) (Tab. 5).

Table 5. Motility of spermatozoa after the collection of semen in the period before the experiment (1—6), during the experiment (7—9: Zindep inj. administration) and after experiment (10—12) between the control and experimental groups

Calendar months	Control group (%)	Experimental group (%)	*t*-test
1–6	70.75 ± 0.44	71.06 ± 0.44	P > 0.05
7–9	70.68 ± 0.59	75.82 ± 0.51	P < 0.001***
10–12	70.69 ± 0.44	72.79 ± 0.35	P < 0.001***

The motility and total concentration of sperms and percentage of surviving sperms decreases in zinc deficient breeding males (4) and as a result fertility is also decreased (17). The motility of sperms is markedly influenced by zinc in the seminal plasma (15). Motility is a better estimator of sperm cell viability than of fertility. The use of ejaculates exhibiting motilities of 60 % or higher in artificial insemination programs should not compromise reproductive performance (8). On the basis of our results the administration of Zindep inj. statistically significantly influenced the motility of sperm after the collection of semen and the motility of sperms in insemination dose 24 hours and 48 hours after dilution.

Statistical significance was not observed in comparing morphological abnormal spermatozoa between the control and experimental groups in the period of experiment (July–September) in the evaluation of pathological forms of head sperms ($P>0.05$), acrosome defects ($P>0.05$), changes of mitochondrial part of flagellum sperm ($P>0.05$), abnormality of shape flagellum ($P>0.05$) and in a number of immature sperms ($P>0.05$). The total number of morphologically abnormal spermatozoa between the control and experimental groups was statistically significant ($P<0.001^{***}$) (Tab. 6).

Morphological changes of sperms cause failure in fertility in cases if they are found in a large number permanently in a certain sire. The percentage of morphological abnormal sperms cannot exceed a limit of 20 % (11, 13). The content of spermatozoa with protoplasmatic droplets should not be higher than 15 %, especially when diluted and preserved semen is used for insemination later after collection and dilution (18). Administration of Zindep inj. did not affect the occurrence of morphological changes in individual parts of sperms but statistically significance was registered in the occurrence of the total number of morphologically abnormal sperms.

Table 6. Differences in the occurrence of morphologically abnormal spermatozoa in boars in the period during the experiment (July—September: Zindep inj. administration) between the control and experimental groups

A period of experiment (July–September)	Control group (%)	Experimental group (%)	*t*-test
pathological forms of head	0.62	0.57	P > 0.05
acrosome defects	1.15	1.04	P > 0.05
changes in mitochondrial part of flagellum	0.13	0.28	P > 0.05
abnormality of shape flagellum	5.15	4.11	P > 0.05
immature sperms	6.59	6.15	P > 0.05
total number of morphological AS	13.02	12.17	P < 0.001***

The number of piglets born per litter, the number of piglets born alive per litter and the number of pigs weaned per litter was not statistically significant between the control and experimental groups of boars (Zindep inj.: July–September) at evaluation in the period of farrowing sows until the time of weaning pigs (farrowing sows: November–January) ($P>0.05$) (Tab. 7).

Table 7. Reproductive indicators of boars evaluated at the time of farrowing sows (November–January) until the time of weaning pigs between the control and experimental groups

Reproductive indicators of boars (November—January)	Control group	Experimental group	*t*-test
average number of piglets born per litter	10.93 ± 0.27	10.63 ± 0.29	P > 0.05
average number of piglets born alive per litter	10.37 ± 0.28	10.26 ± 0.37	P > 0.05
average number of pigs weaned per litter	9.2 ± 0.27	8.9 ± 0.25	P > 0.05

The percentage of fertility of boars after the first insemination was in the control group 82.86 ± 1.26 and in the experimental group 88.33 ± 1.15. The difference between mean values between the control and experimental groups was statistically significant ($P<0.01^{**}$).

The results of fertility are connected with the prolificness of the sows. Fertility is influenced by many factors, primarily the health status of animals, the age and live weight of gilts at the first insemination, nutrition, genetics, influence of external factors of environment (stress), treating animals, daily contact with a boar and a light regime (10). The percentage of fertility under 60 % refers to mistakes in the organization of artificial insemination or on the failure in fertility in breeding. The results in fertility after the first insemination in the range 60–80 % depend on the level of organization of breeding (9).

CONCLUSION

Insufficient saturation of an organism by zinc is possible to solve by revaluing its concentration in the diet and by assessment of its concentration in biological materials. Its total requirements has to take into account all the zinc functions in organism. At the same time we should take note of possible relationships with other microelements, especially with the interaction: zinc – copper.

The results achieved with the preparation Zindep inj. can be used in hypozincaemia arise as a consequence of secondary zinc deficiency, for the purpose of improvement in the quantitative and qualitative parameters of boar semen and for the prevention and therapy of fertility failures in boars.

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IMMUNOSUPPRESSIVE ACTIVITY OF FRACTIONS OF *Salmonella choleraesuis*

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SUMMARY

The aim of the investigation was to determine the influence of fractions of pathogen strain *Salmonella choleraesuis* on the immune response of white mice. The fractions were obtained by gel-chromatography of bouillon filtrate of the strain *Salmonella choleraesuis*, isolated from pig, which had died from acute salmonellossis. The elution curve possesses one peak. It was found, that the fractions from the steep acclivity possesses immunosuppressive properties. These fractions have high molecular weight and probably are lipopolysaccharides. They can suppress both the cell-mediated immunity and the primary and secondary humoral immune response against heterogeneous antigens and phagocytosis. We conclude that the development of septicaemia in *Salmonella* infections depends on the functional condition of the macrophages in the *lamina propria* of the gut wall.

Key words: chromatography; immunosupression; LPS; *Salmonella*

INTRODUCTION

It is known, that some facultative or obligate intracellular pathogens have been shown to suppress the immune response of the infected host (1). According to F l o r o v and B o r i s o v (7) the factors determining this suppressive activity of the pathogen *Shigella* are connected with the O-antigen. On the other hand, such factors have not been detected in the non-pathogen *Shigella*. M o l o z h a v a y a *et al.* (12) have isolated from low-virulent *Salmonella virchov*, a lipopolysaccharide with immunosuppressive activity, which has not been described before.

In this aspect it is important to discover, if the pathogen *Salmonella* also possesses such immuno-suppressive substances and what is its eventual role in the pathogenesis of salmonellosis.

The aim of this study was to evaluate the influence of the fractions of virulent strain *Salmonella choleraesuis* on the immune response of the infected animals.

MATERIAL AND METHODS

Experimental animals. Standard white mice of both sexes with a body weight of 17 to 20 grams were used. Every experimental or control group consists of six animals. The white mice were clinically healthy. During the experiment, they were housed in room conditions.

Bacterial strain. Strain *Salmonella choleraesuis* var. Kutzendorf was used. The strain was isolated from a pig, which had died from acute salmonellosis. The identification of the strain was made by Assoc. Prof. Iv. K a l o j a n o v (National Veterinary Diagnostic and Research Institute, Sofia, Bulgaria).

Fractionization of the bouillon filtrate. The bouillon of the isolated strain (at concentration 2 × 109 cells.ml^{-1}) was centrifuged on 3000 *g* for 45 minutes. The supernatant was passed a trough membrane filter (cut-off 0.32 μm). The fractions of the filtrate were obtained by gel-chromatography on Sephadex

G-150 (column 11 × 500) in the liquid chromatography system – Pharmacia, calibrated with proteins with various molecular weights – human immunoglobulins M and G (IgM and IgG), bovine serum albumin (BSA) and chemotrypsin (Ch). The absorption values were measured at 260 nm.

Testing the immunological activity of the fractions

Cell-mediated immunity. The influence of the fractions on cell-mediated immune response was performed by the method, described by Borisov and Borisova (4). In the white mice there was a delayed type of hypersensitivity against heat-inactivated spleenocytes (100 °C for 30 minutes). The mice were twice injected sub-cutaneously with spleenocytes at a dose of 5.10^7 cells and the second injection was made seven days after the first injection in the left back pad. Simultaneously, the fractions were injected intraperitoneally at a dose of 200 ml. The intensity of the reaction was read 48 hours after the injection as a percentage difference between the masses (mean arithmetical values) of the left (experimental) and the right (intact) sub-knee lymph nodes. As a control, white mice were used, injected only with spleenocytes.

The influence of the fractions on the humoral immune response (primary and secondary) against a heterogeneous antigen was performed on white mice, immunized with ovine erythrocytes.

Primary humoral immune response. The fractions were injected intraperitonealy at dose of one mg protein content simultaneously with ovine erythrocytes (at dose of $8.5.10^8$ cells).

Secondary humoral immune response. The experiment was performed in the same way as that for the primary humoral immune response, as the erythrocytes were injected twice within 14 days at a dose of 5.10^8 cells. The fractions were injected simultaneously with the second injection of erythrocytes.

The samples from blood sera were received seven days after the last injection. The content of the antibodies against ovine erythrocytes was determined by a haemagglutination test (8). The titers of antibodies were expressed as log 2 [T/10], where T is the reverse quantity of the titer.

Phagocytosis. The influence of the fractions on the phagocytic activity of the recipients' leukocytes was carried out on the white mice. The experimental animals were treated intraperitonealy with the fractions at a dose of 1 mg protein content. The test was performed by the method, described by Friemel (8). The blood samples were collected seven days after application of the fractions. We determined the following values: percentage of phagocytosis, phagocyte number and phagocyte index.

Statistic evaluation. The significance of the differences in the values between both groups was assessed by the Student *t*-test.

RESULTS

By gel-filtration on Sephadex G-150, 57 fractions were obtained from the bouillon filtrate of the pathogen strain *Salmonella choleraesuis*. The elution curve possesses one peak with the maximum in fractions № 38—41 (Fig. 1). It was found, that the fractions from the steep declivity (fractions № 18—23) suppress cell-mediated immunity of the treated animals. The injection of these fractions did not cause reliable differences between the mice of the left (experimental) and right (intact) sub-knee lymph nodes. Alternatively, such differences were given in the white mice, treated with other fractions as well in the non-treated animals (control group) (Table 1).

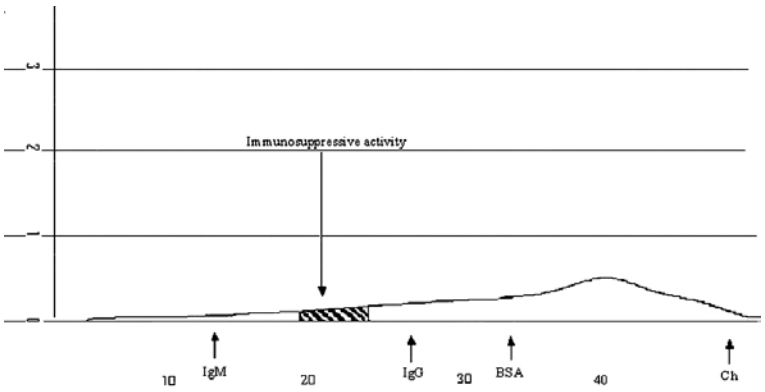
The results from the testing of the influence of the fractions on B-cell immune response are given in Table 1. As can be seen, the same fraction (№ 18—23) can decrease both primary and secondary responses. The serum titres from the mice treated with these fractions are less reliable than these of treated with the other fraction or the untreated mice (control group).

The treatment of the white mice with fractions № 18—23 caused a decrease in phagocytic activity of granulocytes and monocytes. The changes were established in all indices – percentage of phagocytosis, phagocyte number and phagocyte index (Table 1).

Table 1. Immunological indices of white mice treated with fractions of bouillon filtrate of pathogen strain *Salmonella choleraesuis*

Fractions	Cell-mediated immunity*	Humoral immune response**		Phagocytic activity		
		primary	secondary	% phagocytosis	phagocytic index	phagocytic number
1–17	338 ± 19	6.33 ± 1.15	10.16 ± 1.8	16.5 ± 1.32	26.5 ± 0.25	2.05 ± 0.07
18–23	108 ± 7***	3.33 ± 1.70***	6.50 ± 1.6***	11.3 ± 2.75***	19.5 ± 0.1.7***	1.52 ± 0.06***
24–37	166 ± 16****	7.16 ± 1.25	9.83 ± 1.75	15.3 ± 1.43****	28.1 ± 0.31	2.02 ± 0.08
38–43	146 ± 20****	6.16 ± 1.1	11.16 ± 1.50	18.3 ± 1.42	25.8 ± 0.35	2.06 ± 0.09
44–57	330 ± 21	6.50 ± 1.2	9.66 ± 1.8	15.8 ± 1.22	28.5 ± 0.2	1.95 ± 0.1
not treated (control)	420 ± 16	6.66 ± 1.25	10.16 ± 1.75	18.1 ± 1.33	28.2 ± 0.34	2.06 ± 0.05

* — difference (in %) between the masses of the experimental and the intact sub-knee lymph nodes
** — log2 T/10 of the titers against ovine erythrocytes; *** — reliable decreasing the indices compare to control animals ($p < 0.05$)
**** — unreilable decreasing the indices compare to control animals ($p > 0.05$)



Axis X — fractions of the buillon filtrate
Axis Y — optical density of the fractions at 260 nm
Arrows — peaks of elution of human IgG and IgM, bovine serum albumin (BSA) and chemotrypsin (Ch)

Fi. 1. Gel-chromatography of buillon filtrate *Salmonella choleraesuis* through Sephadex G-150. Influence of the gractions on cell-mediated immune responce against heat-inactivated spleenocytes

DISSCUSION

From the above results it follows that the virulent strain *Salmonella choleraesuis* contains substancess with a high molecular weight (lower than the molecular weight of human IgM and higher than the molecular weight of human IgG) which possess immunosuppressive properties. In our view, these substances are of a lipopolysaccharide nature, because they are related by molecular weight to other immunosuppressive substances, isolated from other Gram-negative bacteria, which are known as lipopolysaccharides (5, 7, 12).

The immunosuppressive activity of these substances is manifested by:

a) Suppression of cell-mediated immunity. A similar effect has been established also in the low virulent *Salmonella* (*S. virchov*) and other Gram-negative bacteria. Consequently, suppressing cell-mediated immunity is a universal manifestation of the immunosuppressive action of Gram-negative bacteria.

b) Suppressing both the primary and secondary B-cell immune response. Similar results have been reported by M o l o z h a v a y a *et al.* (12) testing low virulent *Salmonella*. Alternatively, B o r i s o v a (6) has found that pathogen *Shigella* could suppress only the secondary B-cell immune response (immune memory) against thymus-dependent antigens. Therefore, there are some differences between immunosuppressive substances in *Salmonella* and *Shigella*.

c) Suppressing phagocyte activity.

The pathogenic mechanisms of *Salmonella* infection are various and include an effect on both endotoxins and enterooxins (specific exotoxins) (10). However, during septicaemia, *Salmonella* always infects macrophages in the gut wall. This fact corresponds with our discovery, that virulent *Salmonella* possess substances, which can suppress the phagocytosis. Proceed from this, we presume that the immunosuppression is the main pathogenic mechanism of the *Salmonella* infection (probably also in other infections with similar bacteria). This assumption is supported by: firstly, *Brucella suis* infection in pigs is also accompanied with suppression of phagocytosis (3).

Conversely, we found that the treatment of white mice with anti-*Salmonella choleraesuis* DLE leads to a diffuse proliferation of activated macrophages into the *lamina propria* in the gut wall (in the place of *Salmonella* penetration) (2). These macrophages build up a peculiar defensive barrier and protect the treated experimental animals effectively against experimental oral infection. Supporting this are the data of M i k u l a and Š n i r c (11). According to them, treatment with specific DLE leads to an increase in phagocytic activity of peritoneal leukocytes against *Salmonella*.

To conclude, our results demonstrate that the development of the septicaemia in salmonellosis depends on the functional activity of the macrophages in gut wall. Their suppression facilitates their infection by the salmonellas. Similar insights have been expressed by A l - R a m a d i *et al.* (1).

On the other hand, their activation (as a result of cell-mediated immune response) prevents the development of septicaemia. This corresponds to K a t s h u h i k o and T o s h i l i k o (9), according to them the immunosuppression of *Salmonella* is caused by disturbing the interaction between IL-2 and its receptors in murine spleen lymphocytes. It is known that IL-2 is an important mediator of cell-mediated immunity, phagocytosis and thymus-dependent B-cell immunity.

Alternatively, the treatment of experimental animals with a transfer factor causes an expression of IL-2 receptors on the surface of the T-cells (in all populations) – M i k u l a and Š n i r c (11). In this case, the transfer factor is an antagonist of these suppressor substances.

Logically, the following question arises: are not these two phenomena inconsistent? On the one hand *Salmonella* can oppress cell-mediated immunity, but on the other hand they are good inducer of the transfer factor (2, 11).

According to us, these two phenomena (apparently opposed) do not contradict each other, because they develop into different phases in the cell-mediated immune response. The transfer factor is synthesised earlier and this synthesis is independent of the interaction between IL-2 and its receptors.

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THE CAST METAL CROWN WITH INTEGRATED POST UNIT – THE ROLE OF POST LENGTH AND STUMP BEVELLING ON CANINE TOOTH STABILITY IN DOGS

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ABSTRACT

Our study evaluates the effect of the length of the endo posts (1.5 or 2 mm) and height of crown bevelling (2.4 and 6 mm) of the reconstructed crowns against pressure. The study was carried out on eleven skeletonized skulls of German Shepherd dogs. Zinc phosphate cement (ADHESOR, Dental Praha) was used to fix the cast metal crown and post unit in the prepared canal. The compressive forces were measured in Newtons (N) employing a mechanical tensile testing machine up to 10 kN, type FM 100, manufactured by VEB Thüringer Industriewerk Raunstein. The results obtained allow us to draw the following conclusions: the use of the crown attachment zone of 4 mm provides the maximum increase in crown strength; the longer post generally does not provide increased stability against pressure. The crown attachment zone represents the most stable element of an integrated cast metal crown and the post unit if its height exceedes 2 mm. Another stabilizing factor is the length of the cast metal post, but its importance also depends on the dimensions of the tooth. A long post may decrease the stability of restoration in smaller canines.

Key words: canines; dog; fracture; metal crown

INTRODUCTION

Tooth fractures are frequently encountered in dogs due to the action of abnormal occlusive forces or various traumas in the orofacial region. In dogs which have been anaesthetised for other than dental reasons Golden *et al.* (4) have observed as high as a 27% incidence of fractured teeth. In the recent period we have observed an increasing number of cases involving tooth fractures.

If only one canine tooth is fractured biting may become unbalanced and cause fracture of an opposing tooth or contralateral canine. Due to fractured teeth the performance of these dogs can deteriorate considerably as described by Beard in 1991 (1). Progressive dental treatment techniques that have emerged over the two past decades may also be used successfully in veterinary practice.

As a dog's bite is very powerful, strong reconstruction materials have to be used. Linder *et al.* (5), have recorded that the requirements are best met by full metal crowns. In show dogs suitable choices are ceramic-metal, resin-metal or full resin crowns, eventually only composite build-up with strengthening posts, because these dogs do not strain their teeth as intensively as the dogs kept as household pets, as Remees has claimed (8).

In dogs of working breeds the forces acting on canine teeth represent a combination of compressive, tensile, cutting and rotation forces. In such cases the preparation of a stump does not necessarily ensure good bonding with the crown and subsequent resistance to strain and therefore good cement bonding is very important.

Routine cementing materials do not produce bonds of sufficient strength therefore it is necessary to consider carefully their use in working dog breeds. According to White and Zhaokun (10) better properties in this direction are

ensured by silicon (so-called glass ionomer) cements which combine good retention forces with relatively high tensile strength upon straining.

The type of prosthesis most frequently used in veterinary dentistry is the crown prosthesis used to restore damaged crowns (usually metal cast crown). In general, crown prosthesis is indicated for simple damage to one tooth. This most frequently concerns canine teeth, fourth maxillary premolars and sometimes also incisors. A removable prosthesis (partial removable prosthesis, complete dental prosthesis) is rare in veterinary dental prosthetics. Most frequent are cast crown prostheses intended for individual teeth. Full crowns are most common but it is also possible to use partial crowns or half-crowns.

MATERIAL AND METHODS

The study was carried out on eleven skeletonized skulls of German shepherd dogs. The mandibles and maxillae were impregnated with paraffin oil to prevent the drying of hard tooth tissues which could increase their fragility and brittleness. Canine tooth crowns were shortened to the level of the incisal edge of the third incisors (i.e. the level of crown stump which results in complete loss of function of this tooth).

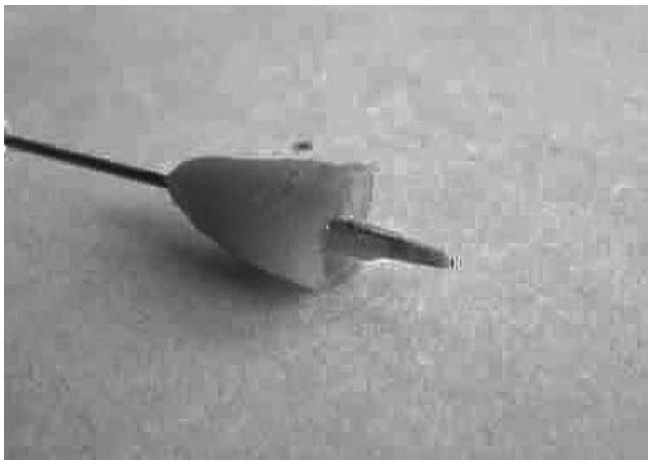

Fig. 1. Model of the individual crown prepared by direct method from a dental wax

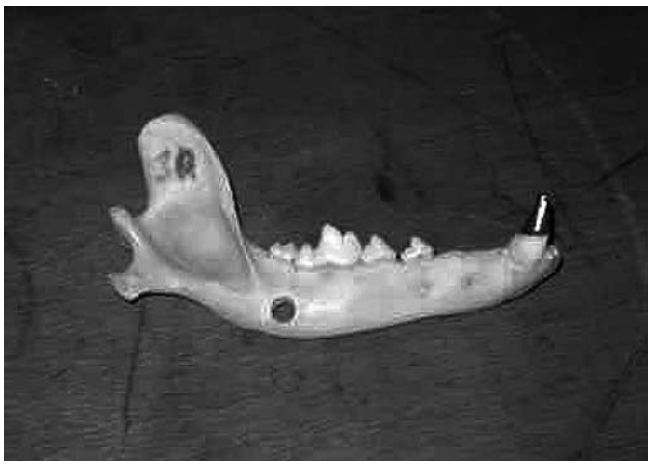

Fig. 2. Mandibular canine tooth crown stump reaching the level of the third incisors (the loss of canine function)

Despite using canine teeth from one breed the size of teeth varied. The crown width at the level of alveolar crest ranged between 0.7 and 1 cm and the mesio-distal distance between 1.1 and 1.5 cm. In order to compare the influence of individual factors and to eliminate the size differences we used canine teeth pairs originating from the same individual.

Canals for endodontic posts were first pre-drilled with a cylindrical flat face dental bur inserted in a high speed handpiece to a depth of approximately one centimetre in the direction of the root canal. The remaining cavity of the required depth was prepared by a low-revolution drill using a cylindrical dental bur. During the drilling, the preparation instruments and tooth tissues were protected against thermal damage by cooling with air and a water gun. We tried to spare the hard tooth tissue to the maximum possible degree. The shape of the cavity prepared in this way was oval in the mesio-distal direction in order to eliminate the torsion of the crown post due to the action of compressive forces. The enamel of the crown stumps in the second group was removed in a range of two to six millimetres.

Models of individual crowns were prepared by a direct method. In order to prepare a model of the crown post prosthesis we used carving wax sheets (Fig. 1). Metal cast crowns were prepared from chromium-cobalt alloy (REMANIUM) in a dental laboratory.

Zinc phosphate cement (ADHESOR, Dental Praha) was used to fix the post of crown in the prepared root canal (Fig. 2). It was also necessary to fix the canine teeth in the alveolar socket by means of the zinc phosphate cement to replace the missing periodontal ligament and eliminate the increased movement of the tooth in the tooth socket during the measurement of compressive forces.

The compressive forces were measured at the Technical University in Košice employing a mechanical tensile testing machine up to 10 kN, type FM 100, manufactured by VEB Thüringer Industriewerk Raunstein (Fig. 3). We recorded the force in Newtons (N) necessary to fracture the reconstructed tooth. Because the alveolar bone in the maxilla undergoes destruction before the reconstructed maxillary canine teeth, these teeth were fixed in methyl metacrylate blocks (DURACOL, Dental Praha) in such a way that they were in a position cor-

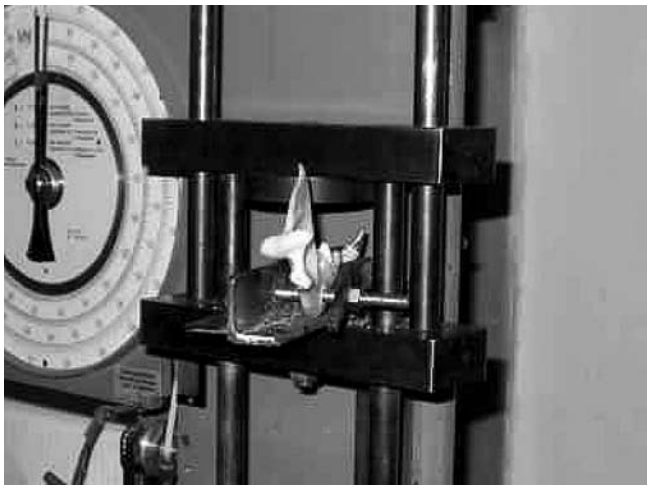

Fig. 3. Mechanical tensile testing maschine

responding to their anatomical situation in the maxilla. The mandible was fixed in a fixing apparatus prepared beforehand. Twelve intact canine teeth (six maxillary, six mandibular) were used as a control group.

The aim of the study was to evaluate the effect of the following factors on the strength of the reconstructed crowns:

1. length of the endodontic post-16 mandibular canines (length of the pin: Ist group – 1.5 cm, IInd group – 2 cm);

2. height of the crown attachment zone-16 maxillary canines (the height ranging between 0.2—0.6 cm).

The influence of the height of the crown attachement was statistically evaluated using CHI-test.

RESULTS

Integrated cast metal crown and post units of height reaching the original crown height were affixed to 32 maxillary and mandibular canines by means of zinc phosphate cement. Twelve intact canine crowns (six mandibular, six maxillary) were used as a control. A pressure force of 490—670 N was necessary to cause the fracture of intact mandibular canines while the maxillary canines fractured at forces ranging between 3,200—3,800 N.

The group of canines with varying post length: 16 teeth

When shorter posts (fifteen millimetres) were used, the mandibular canine crowns fractured at pressure forces ranging between 300 and 500 N. Cast metal crown and post units in mandibular canines with posts of length reaching twenty millimetres withstood pressure forces of 310 to 620 N (Fig. 4).

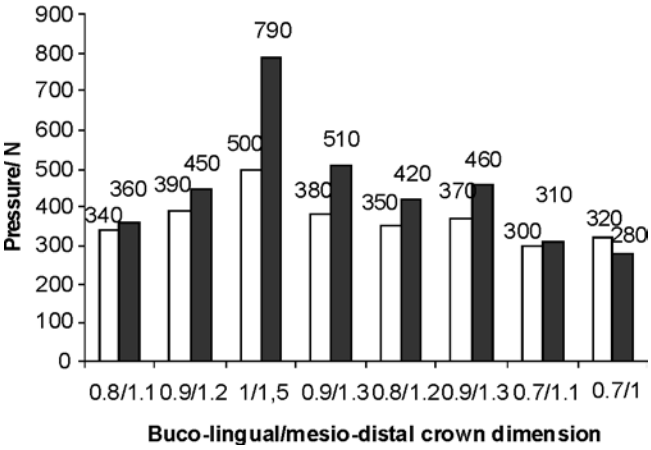

Fig. 4. Post length: pressure force relation

The group of canines with varying crown attachment zone: sixteen teeth

Cast metal crown and post units of maxillary canines equipped with bevelled crown stump resisted to forces ranging between 3,400 and 10,000 N (Fig. 5). When the same direction and length of post but different crown

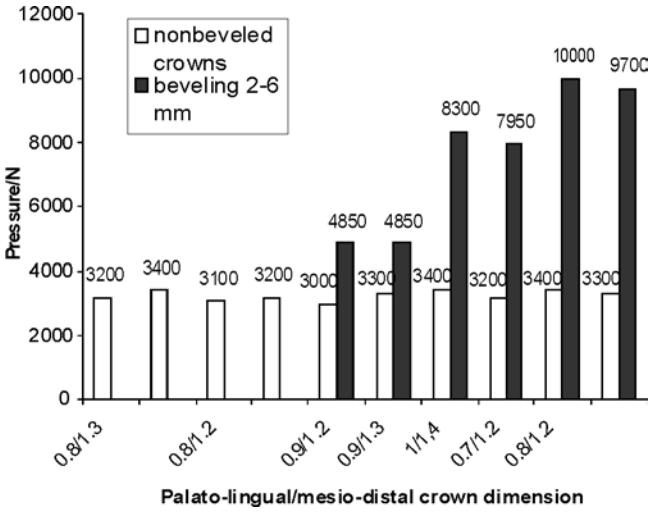

Fig. 5. Compressive force: crown bevelled height

attachment zone was used the following values were measured in maxillary canines:

– maxillary canines without attachment zone (No. 1, 2) resisted to pressure forces in the range of 3,000 to 3,400 N,

– maxillary canine teeth with crown attachment zone of 2 mm (No. 3, 4) – 4 850 N,

– maxillary canines with crown attachment zone of 4 mm (No. 5, 6) – 8 300 N,

– maxillary canines with crown attachment zone of 6 mm (No. 7, 8) – > 10 000 N.

Statistical analysis using CHI-test is expressed in the Fig. 6.

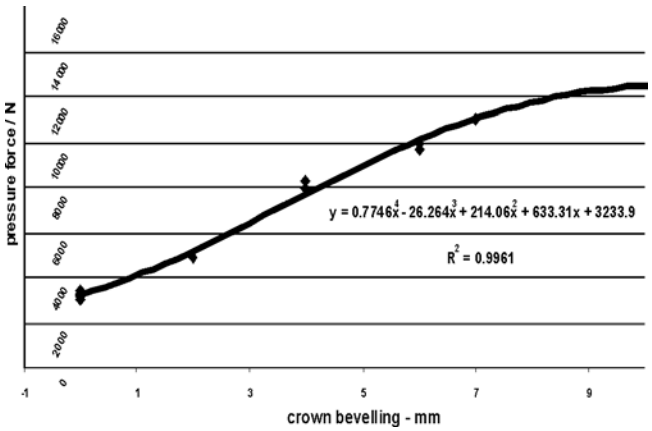

Fig. 6. Relation pressure force to crown bevelling-trend line (CHI-test)

DISCUSSION

A reduced crown height frequently results in a reduced biting force and functional deficit. Pavlica and Lukman (7), recommend for dogs of working breeds the strong reconstruction of canine teeth so they can resist external forces associated with their perform-

ance. A shorter canine tooth may nor represent an obvious problem when the remaining tooth crown portion is sufficiently high and the crown stump is undamaged. However Pavlica (6), has stated that there always remains some risk of recurrent fracture of the respective canine tooth or other canines because of the loss of physiological balance of the bite which is otherwise ensured by canines of equal size.

According to Fichtel (3), one of the preconditions for successful reconstruction of fractured teeth is the good health status of the periapical tissues, which are frequently affected, particularly as a result of long-lasting conditions.

Borrisov (2), has stated that at least one third of the coronal (i.e. the stump should be no lower than one third of the original clinical crown) tooth portion should be available for successful crown therapy. It is claimed, that a successful canine tooth crown prosthesis requires preservation of a minimum of two thirds of the coronal tooth part if cementation is to ensure good crown retention. If the crown stump is shorter than two thirds of the original clinical crown, it is necessary to build-up the core or the core and pin to increase retention.

The data on the long-term results of the application of canine teeth crown prosthesis in dogs of working breeds are scarce. The studies published up to this date mostly lack long-term follow-up information.

By evaluating the measurements carried out on pairs of maxillary or mandibular canines originating from one dog we eliminated the influence of varying tooth size between individuals of the same breed on the magnitude of pressure forces. The width of the canines at the fracture surface (bucco-lingual/palatal dimension) ranged between 0.7—1 cm and the length of the fracture surface (mesio-distal dimension) was in the range of 1.1—1.5 cm.

The forces measured, particularly those applied to maxillary canines, cannot be considered as absolute forces which the teeth *in vivo* could sustain. Fixation in methyl metacrylate produced a strong anchorage of the tooth, which increased its strength and necessitated the use of bigger compressive forces to produce a crown fracture. Our study did not focus on the determination of maximum compressive forces, which the restored canines could withstand, but on expression of ratios of these forces under the influence of factors described in Material and Methods.

When comparing the magnitude of compressive forces one must take into account the different anatomical localization of canines in the mouth cavity (more vertical position of maxillary canines versus rostral-lateral orientation of mandibular canines). These ratios result in a more marked influence of leverage and torsion forces on mandibular canines resulting in fractures at lower forces. Linder *et al.* (5), described the magnitude of bite forces in working dogs, which can produce a force up to 1394 N.

To verify the tooth strength *in vitro* we used twelve undamaged canines. Maxillary canines resisted forces from 3,200 N to 3,800 N and mandibular ones to forces from 490 to 670 N.

Our observation of the action of pressure forces on intact canines indicated the different direction of forces acting on mandibular and maxillary canines manifested by a different course of fracture surface. The fracture surface in maxillary canines was located in the mesial part of the crown and run vertically. Mandibular canines showed a shift of the fracture surface towards the distal crown edge with dorso-mesio distal-ventral running. These differences in orientation of the fracture surface confirmed that application of compressive forces to the apical end of the crown of mandibular canine results in division of this force to compressive, leverage, and even torsion forces at certain ratios.

It has been assumed that the length of the endodontic post is related directly to the crown strength, i.e. the longer the post the higher the crown strength. Our results did not generally confirm this assumption. The extent of compressive forces is a product of size differences in canine teeth. Pavlica and Lukman (7), have stated that the post itself is not a guarantee of sufficient stability. Our observations point to the fact that, despite sufficient post length, crown strength can be diminished considerably. The factors that affect the results include: 1) tooth dimensions and 2) the loss of crown mass at the fracture surface. In small canines long endodontic posts with tips closer to the apex decrease tooth resistance against compressive force. In the case of loss of tooth tissue of the crown stump in the mesial part of the crown and the respective cast metal crown and post build-up, significantly lower values of compressive forces were measured.

The results presented have confirmed that the compressive forces acting on reconstructed canines act particularly on the mesial part of the crown stump. Any weakening of the crown stump mesial part decreases the stability of the reconstructed crown considerably. Fractures of this character are typical of the action of tensile forces (i.e. acting in a rostral direction). If the crown stump surface is reduced in the mesial part, the pressure force is distributed over a smaller surface resulting in fractures of the crown stump caused by smaller forces. It is therefore important, if permitted by the course of the fracture surface (i.e. if the fracture does not extend beneath the gingival edge), to ensure the maximum possible width of the coronal surface of the broken canine crown stump even at the expense of the crown stump height.

Crown stability can be increased by certain stabilising elements, such as stump beveling. Our investigations indicated considerable increase in the strength of bevelled crowns (four millimetres high). In such cases we observed the highest increase in resistance of canines to such forces. Crowns with beveling two millimetres high showed an increase in strength by 53.9 % in comparison with nonbevelled crowns. The bevelled height of four millimetres resulted in a strength increase by 146.2 % and the height of six millimetres in 194 % increase. These

indicate that resistance increased arithmetically with increasing facet height up to four millimetres. However, an additional increase in facet height failed to result in a regular increase of resistance to compressive force. We explain this by the fact that despite strengthening through facet use the natural dental tissue is unable to resist to greater forces.

ACKNOWLEDGMENT

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RESISTANCE OF POULTRY, SWINE AND HUMAN STRAINS OF *Campylobacter jejuni* AND *C. coli* TO SELECTED ANTIBIOTICS: A COMPARATIVE STUDY

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ABSTRACT

Between 2003 and 2005, *Campylobacter coli* and *C. jejuni* strains isolated from pigs, poultry and humans were tested for antibiotic resistance by the agar dilution method. The following antibiotics were tested: ampicillin, tetracycline, erythromycin, nalidixic acid, ciprofloxacin, gentamicin and chloramphenicol. *C. jejuni* strains from pigs demonstrated a high prevalence of resistance to ciprofloxacin (54 %), erythromycin (56 %) and tetracycline (62 %). A majority of poultry strains of *C. coli* (76 %) showed resistance to nalidixic acid and ciprofloxacin (73 %). Compared with swine *C. coli* strains, the prevalence of resistance to erythromycin and tetracycline was substantially lower. In human strains of *C. coli*, the highest prevalence of resistance was demonstrated in the case of ciprofloxacin (49 %) and nalidixic acid (30 %). Resistance prevalence characteristics of human and poultry strains of *C. jejuni* showed many similarities. A majority of poultry strains of *C. jejuni* were resistant to ciprofloxacin (69 %), nalidixic acid (23 %) and ampicillin (19 %). The highest prevalence of resistant human *C. jejuni* strains was found in the case of ciprofloxacin (44 %), followed by ampicillin (28 %) and nalidixic acid (20 %). The prevalence of resistance among *C. jejuni* strains isolated from pigs is different. These strains show a low resistance prevalence particularly to fluoroquinolone antibiotics (nalidixic acid and ciprofloxacin), but very high resistance prevalence to erythromycin, which, among poultry and human strains of *C. jejuni* is quite infrequent. Resistant strains of *C. coli* and *C. jejuni* are found relatively frequently among food animals.

Key words: antibiotic resistance; *Campylobacter* spp.; occurrence;

INTRODUCTION

Campylobacteriosis ranks among the most frequent diseases of alimentary origin. Also in many industrial European countries the rate of *Campylobacter* spp. infections has been increasing, with the number of cases often exceeding those of *Salmonella* and *Schigella*. Although most laboratories do not routinely perform species identification of causative agents, *C. jejuni* is believed to be the leading cause of human food borne gastroenteritis, while *C. coli* is suspected in only a few occurrences (1).

Campylobacter spp. are present in the gastrointestinal tracts of many animals without any clinical symptoms of the disease in those animals. Wild birds and poultry are very frequent carriers of *C. jejuni*. The prevalence in poultry is estimated at 60 % to 80 %, but, according to some authors, 100 % prevalence of *Campylobacter* spp. in chickens and especially turkeys is frequent. The positive findings in pigs are relatively frequent, but in their case, *C. coli* isolates predominate. Similarly in other farm animals, e.g. sheep and cattle, the incidence of *C. coli* is also no exception and is closely connected with the quality of herd management and hygienic conditions (2).

Running head
Antibiotic resistance of *Campylobacter jejuni* and *C. coli*

The use of antimicrobial preparations in primary production in agriculture has resulted in the emergence of resistant strains of pathogenic microorganisms, including *Campylobacter* spp. Having at first gradually limited their use, EU member states have now banned the use of antibiotics in feed mixes for food animals completely (3). In spite of the ban on the administration of antibiotics, resistance of pathogenic micro-organisms is reported by many countries and long-term surveillance is under way there. In the Czech Republic systematic monitoring of *Campylobacter* spp. resistance is not obligatory and, until recently, no method had been established for the evaluation of *Campylobacter* spp. resistance. For that reason our department has optimized a method for the evaluation of the resistance of *Campylobacter* spp. strains from poultry and pigs, and has tested it for several years. The aim of the study was to compare the antibiotic resistance of *C. jejuni* and *C. coli* strains from pigs and poultry, and compare it with the resistance rates of human strains.

MATERIAL AND METHODS

Samples were obtained as smears from the surface of poultry and pig carcasses in abattoirs. Human strains were obtained from patients suffering from gastroenteritis, and were tested in cooperation with Státní zdravotní ústav Praha and Bioplus s.r.o. For examinations, cultivation techniques and biochemical tests according to ČSN ISO 102 72 were used. Species identification and typing of thermofilic strains of *Campylobacter* spp. were performed using the PCR/RFLP method (4).

The sensitivity of *C. jejuni* strains to selected antimicrobial substances was tested by the agar dilution method. In this method, the minimum concentration of an antibiotic necessary for the growth inhibition of the bacterial cells studied is determined. The NCCLS M11-A6 (5) document gives the minimum inhibitory concentrations (MIC) of ampicillin (8—32 $\mu g.ml^{-1}$), tetracycline (≥ 40 $\mu g.ml^{-1}$), nalidixic acid (≥ 32 $\mu g.ml^{-1}$), chloramphenicol (≥ 32 $\mu g.ml^{-1}$), erythromycin (≥ 8 $\mu g.ml^{-1}$), ciprofloxacin (≥ 1 $\mu g.ml^{-1}$) and gentamicin (≥ 4 $\mu g.ml^{-1}$). The antibiotics were diluted to final concentrations of 128; 64; 32; 16; 8; 4; 2; 1; 0.5 and 0.25 $mg.l^{-1}$, ciprofloxacin and gentamicin concentrations were 32; 16; 8; 4; 2; 1; 0.5; 0.25; 0.125; 0.063 $mg.l^{-1}$. As controls, collection strains *Campylobacter coli* ATCC 43478 and *Campylobacter jejuni* subsp. *jejuni* ATCC 33560 (FCCM, Prague, CR) were used. After microaerophilic incubation of the samples, sensitivity was evaluated according to NCCLS M11-A6 (5).

RESULTS

Between 2003 and 2005, 97 *C. coli* strains from **pigs** were tested for antibiotic resistance. A high prevalence of resistance to tetracycline, ciprofloxacin and erythromycin was found. Resistance to tetracycline was demonstrated in 60 strains (62 %). Resistance to ciprofloxacin was found in 52 strains (54 %), and 54 (56 %) strains were evaluated as resistant to erythromycin. One third of the strains were resistant to nalidixic acid. Ampicillin resistance was low (20 %), and we were practically unable to identify any *C. coli* strains resistant to other antibiotics (i.e. gentamicin and chloramphenicol). Resistance of *C. jejuni* strains from pigs was similar: highest to tetracycline (38 %), erythromycin (33 %), and ampicillin and ciprofloxacin (29 %). Only a low prevalence of resistance to nalidixic acid was found, and none of the *C. jejuni* strains isolated were resistant to gentamicin or chloramphenicol (Fig. 1).

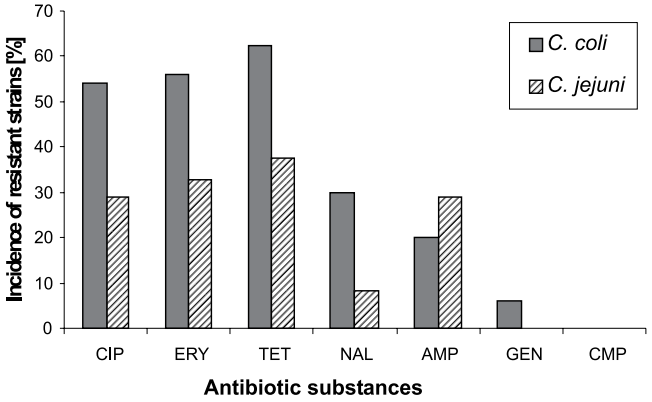

Fig. 1. Antimicrobial resistance of pig strains *C. coli* and *C. jejuni*

In the study, we examined a total of 55 strains of *C. coli* from **poultry**. A majority of the strains (76 %) were resistant to nalidixic acid, and 40 strains (73 %) were ciprofloxacin resistant. Only five (9 %) of the strains isolated were resistant to erythromycin and eleven (20 %) strains were tetracycline resistant. At the same time, however, 30 strains (55 %) tested ampicillin resistant, which is a much higher interception rate than that found in swine *C. coli*. Resistance characteristics of *C. jejuni* strains from poultry were rather different. Most of them were resistant to ciprofloxacin (69 %), and only 23 % to nalidixic acid. Resistance to other antibiotics has similar profile as resistance of *C. coli*. Three of the *C. jejuni* strains isolated showed resistance to chloramphenicol (Fig. 2).

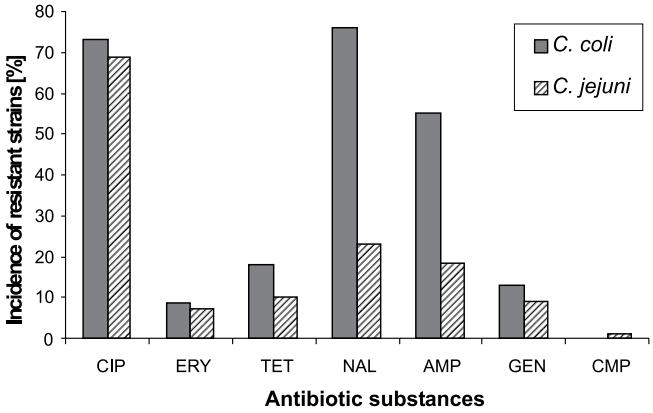

Fig. 2. Antimicrobial resistance of poultry strains *C. coli* and *C. jejuni*

Seventy-seven **human strains** of *C. coli* were also examined. The highest frequency was demonstrated in resistance to ciprofloxacin (49 %), and to nalidixic acid (30 %). About a quarter of the human strains were resistant to ampicillin and erythromycin. Resistance characteristics of human *C. jejuni* strains were similar to those isolated from poultry. The highest prevalence of resistance was found in the case of ciprofloxacin (44 %), followed by ampicillin (28 %) and nalidixic acid (20 %). Only three of the *C. jejuni* strains isolated were resistant to gentamicin and eleven strains to erythromycin. None of the strains were resistant to chloramphenicol (Fig. 3).

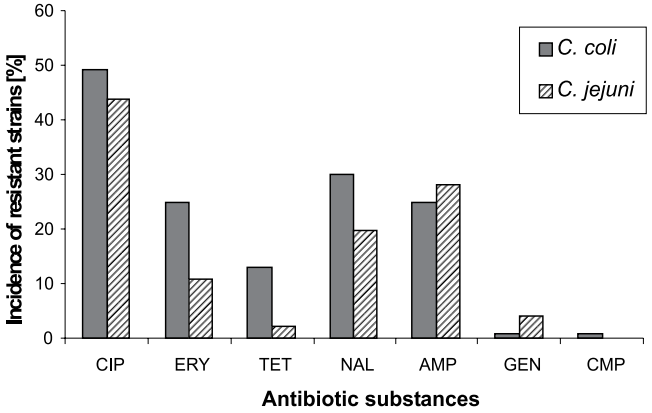

Fig. 3. Antimicrobial resistance of human strains *C. coli* and *C. jejuni*

It follows from the results of our study that the prevalence of resistance among *C. coli* strains from poultry is substantially higher than that among swine strains. While resistance to nalidixic acid and ciprofloxacin was demonstrated in three-quarters of the poultry strains, resistance to erythromycin and tetracycline was substantially less prevalent.

Although the resistance characteristics of human and poultry strains of *C. jejuni* showed many similarities, the resistance of poultry strains of *C. jejuni* to ciprofloxacin is substantially higher. The prevalence of resistance among *C. jejuni* strains from pigs is considerably different. It is low mainly to fluoroquinolone antibiotics (nalidixic acid and ciprofloxacin) compared with human and, especially, with poultry strains. At the same time, however, swine strains of *C. jejuni* show a high prevalence of resistance to erythromycin, which, on the contrary, is infrequent among poultry and human isolates.

DISCUSSION

Antimicrobial agents are used in veterinary medicine primarily for therapeutic purposes. Administering preventive doses of antibiotics for prophylactic purposes or growth promotion is a widespread practice, in which cases antibiotics not registered for treatment in veterinary or human medicine are used (6). It is this type of antibiotic use that may produce a selection pressure on micro-organisms and thus lead to the emergence of large numbers of resistant strains. For that reason the use of agents with antimicrobial action for other than therapeutic purposes is prohibited in the EU.

The prevalence of resistance is generally higher among *C. coli* strains than *C. jejuni* strains, and this is particularly true of resistance to macrolides especially among *C. coli* strains isolated from pigs. Similar to our results, a very high prevalence of erythromycin-resistant swine strains has been determined in the UK, where 84 % of strains were reported resistant in 2001. Two thirds of ciprofloxacin-resistant isolates were also resistant to erythromycin (7). The incidence of resistance among these strains to macrolides and fluoroquinolones is generally high, while resistance to other antimicrobial agents including tetracycline, amyloglycosides and chloramphenicol is relatively low. Resistance to beta-lactam antibiotics and sulphonamides among *C. coli* strains is also generally very high (8).

Resistance to macrolide antibiotics is based on a different mechanism, namely that of a specific methylation of adenins of the target site of macrolides at 23S rRNA, which may be genetically determined (by mutation). The high prevalence of resistance to macrolide antibiotics among swine strains of *C. coli* is probably due to a high selection pressure on bacteria that is produced, e.g., by the use of tylosin as a growth promoter. For that reason this practice has been banned since 1999 (8).

Besides resistance to macrolides, *Campylobacter* spp. are often reported resistant to quinolone antibiotics. Fluoroquinolone enrofloxacin was first used in veterinary medicine in 1987, and no *Campylobacter* sp. strains resistant to it were reported until the early 1990s. In 1992, 29 % fluoroquinolone-resistant strains were reported in poultry in the Netherlands, and resistance among human strains was first discovered in 1997. A similar situation has been observed in Austria, Denmark, Finland, France, Italy, Spain, UK and the USA (3).

Fluoroquinolone antibiotics inhibit activity of DNA gyrasis, and a majority of bacterial strains has gained resistance thanks to mutations in the genes of gyrase or topoisomerase. Fluoroquinolone resistance of *Campylobacter* spp. strains is caused by mutations in gyrA gene at positions Thr-86, Asp-90 and Ala-70 (7).

Considerable numbers of *C. jejuni* and *C. coli* strains show resistance to beta-lactam antibiotics thanks to their ability to produce beta-lactamase. Another mechanism employed by a large number of ampicillin-resistant strains is the low capability of antibiotic binding to suitable proteins and a low cell permeability for the antibiotics (8).

High resistance prevalence to ciprofloxacin (69 %) and nalidixic acid (23 %) was found among our *C. jejuni* strains, particularly those isolated from poultry. Similarly high rates of *C. jejuni* resistance to ciprofloxacin (44 %), nalidixic acid (44 %) and tetracycline (34 %) have been reported from, e.g., Belgium. In the late 1980s, a number

of quinolone-based preparations were approved for veterinary uses (flumequin, enrofloxacin, difloxacin). The appereance of highly resistant strains is often traced to the use of these agents for therapeutic use in poultry (9). A similar emergence of ciprofloxacin-resistant strains has been reported from Spain, where the prevalence of resistance increased over a period of only two years from 0 to 3 % in 1989 to 30 to 50 % in 1991. This increase has been related to the administration of enrofloxacin for veterinary uses (10). Administration of sarafloxacin and enrofloxacin also increased resistance rates in the USA between 1996 and 1998 (11).

Resistance to macrolide and fluoroquinolone antibiotics is caused by chromosome mutations rather than by horizontally transmitted genes. Some findings, however, suggest the possibility of resistance transfers between individual bacterial species (8).

The course of the majority of diseases caused by *Campylobacter* spp. in patients with properly functioning immune system is mild, and no antibiotics are needed. Because, however, there are more and more less well functioing patients, the number of cases when antimicrobial preparations need to be administered at the onset of the disease is also increasing. And macrolides (erythromycin) and fluoroquinolones (ciprofloxacin) are the most frequently used antibiotics of choice. Just they are the antibiotics that used to be administered to food animals on a massive scale in the past (7).

Rather than reducing the pathogen prevalence on poultry and pig farms, the use of antibiotics has produced resistant strains there. The ban on the use of agents with antimicrobial action for other than therapeutic purposes in the EU will hopefully improve the situation in the foreseeable future.

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SENSORY, TEXTURAL, AND PHYSICO-CHEMICAL PARAMETERS OF HEAT-TREATED DURABLE MEAT PRODUCT VYSOCINA

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ABSTRACT

Correlations of the sensory, textural and physicochemical parameters of a heat-treated durable meat product were evaluated. We chose Vysocina (Czech sausage) as a model subject. Samples of the sausage from three different manufacturers were taken from the retail network over a thirteen-week period. We monitored chemical parameters such as the content of NaCl, dry matter, ash, pure muscle proteins, collagen and fat. At the level of significance $P \leq 0.05$, there was no variation in the content of dry matter, pure muscle proteins and collagen present among different producers. Sensory parameters were chosen according to the Czech legislative requirements – appearance of the cut surface, colour, matrix, odour, consistency, texture and taste.

At the level of significance $P \leq 0.05$, manufacturers did not differ in the evaluation of colour, matrix and texture. Subsequently, we generated a correlation matrix and calculated correlation coefficients. The content of pure muscle proteins, which correlated with the other five parameters within the correlation coefficient limits 0.39 to 0.47 at the level of significance $P \leq 0.05$, appeared as the most important indicator. We found a relationship to exist between collagen content determined chemically, texture defined based on sensory evaluation and shear force measured instrumentally.

Key words: dry cooked sausage; sensory evaluation; shear force; texture

INTRODUCTION

The quality of food products, in conformity with consumer requirements and acceptance, is determined by their sensory attributes, chemical composition, physical properties, and level of microbiological and toxicological contaminants, shelf life, packaging and labelling (4, 1).

When characterizing products typical of a specific region or country, physico-chemical parameters (pH, water activity, content and composition of fats, proteins and salts), sensory analysis (10, 5) as well as instrumentally measured parameters such as colour and texture (2) are used.

Consumer selection puts pressure on producers to reduce the content of fat in meat and meat products, which negatively affects the textural properties of meat (9). Besides texture, a lower fat content also affects the taste of meat products negatively. When fat content is reduced by increasing the proportion of water added and keeping the amount of protein essentially constant, low-fat products can be obtained with less hardness (8).

The tendency to reduce fat content is also evident in Czech legislation, which has reduced the maximum permissible amount

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of fat in some meat products, e.g. Vysocina, from a previous 55 percent of weight (% wt) to 50 % wt (11, 12). Despite the relatively high fat content these meat products are still very popular and have a long-standing tradition.

According to Czech legislation (11), Vysocina ranks among heat-treated durable meat products, dry cooked sausages, respectively, which means that thermal effect corresponding to + 70 °C temperature operating for 10 minutes must be reached in every part of the product during the heat treatment. During the process of smoking and drying, water activity decreases below a value of 0.93 and the product then attains a minimum shelf life of 21 days at + 20 °C temperature. The Czech legislation imposes strict requirements for Vysocina with respect to the raw materials used (the principal raw material to produce the sausage is beef and pork meat), chemical parameters (content of fat and pure muscle proteins), and sensory quality. In particular, considerable attention is paid to sensory quality. The legislation has established requirements for consistency, appearance on the cut surface, matrix, odour, and taste (11).

The aims of the present paper were to establish a mutual correlation between physico-chemical, sensory and instrumentally measured parameters of the sausage Vysocina (as an example of the dry cooked meat product) and to determine whether there is any statistically significant variation in these parameters between different producers.

MATERIAL AND METHODS

We took samples of the sausage Vysocina from three producers from the retail network in the Czech Republic over a period of thirteen weeks; each week 1 sample from each producer, 39 samples together. The samples were subjected to chemical, sensory and instrumental analysis. All data was statistically processed using STATISTICA CZ 7 software (Statsoft, the Czech Republic).

Sensory analysis

Samples were sliced to 1 cm thick slices and evaluated by a panel composed of twelve members comprising employees and postgraduate students of the Institute of Meat Hygiene and Technology, University of Veterinary and Pharmaceutical Sciences, Brno. The panel was trained with respect to the CSN ISO 8586-1 standard and was introduced to product sensory requirements according to the applicable public notification. Two specimens of each sample were given to each evaluator on an anonymous basis; hence, we obtained 24 evaluations for each sample.

Seven parameters (appearance on the cut surface, matrix, colour, odour, consistency, texture and taste) were evaluated by means of ten-centimetre-long, non-structured graphic scales. The left end of the scale represented the fully satisfactory condition of a parameter, the right end the fully unsatisfactory condition of the same.

The fully satisfactory conditions for the individual parameters were: appearance on the cut surface – very fine mosaic, predominant grain size of raw material 1 mm; colour – deep pink on cut, darker towards the margins; matrix – scarce soft collagen particles, scarce air bubbles; odour – aromatic odour after smoking, without any strange smell, suitably intensive, and smooth with respect to spiciness; consistency – rather firm, cohesive; texture – dense feel on teeth, without notable solid particles; taste – reasonably salty and spicy.

Sensory evaluation was always carried out the second day after the sample purchase while before the analysis the samples were stored at 5 ± 2 °C temperature in the refrigerator and, immediately before the analysis, heated up to 10 ± 2 °C temperature, which is the temperature considered typical at consumption. Rolls were provided to clear the palate between samples.

Instrumental analysis

Instron 5544 (Instron Corporation, England) with a Warner-Bratzler test measuring maximum shear force (N) necessary to cut the sample was used for instrumental texture measurement. Samples with dimensions 2.5 × 2.5 × 1.5 (height) cm were analysed at room temperature. Speed of the crosspiece was set to 80 mm.min^{-1}. Results were represented as a mean value of ten measurements.

Physico-chemical analysis

Samples were homogenised and, subsequently, the following parameters were determined: amount of dry matter in % wt (sea sand drying, 24 hours at 103 ± 2 °C temperature, according to Czech standards CSN 57 6021), ash in % wt (burning in muffle oven, gradual increase of temperature – once reaching 650 °C, the temperature was kept at this value for six hours), chlorides in % wt (Volhard method, according to Czech standard CSN ISO 1841-1), fat (analysed instrumentally on SOXTEC instrument, producer TECATOR, diethylether was used as extraction agent), collagen (spectrophotometry at 550 nm, equalized to 4-hydroxyprolin content) and pure muscle protein (after elemination of non-protein N-compounds by hot tanine, protein content was set on KJEHLTEC, producer TECATOR, pure muscle protein was calculated as protein content reduced by the amount of the collagen).

Results were taken as a mean value of three parallel measurements of one sample.

Statistical analysis

Results were processed using the STATISTICA CZ 7 program (Statsoft, Czech Republic). In order to determine the covariance we generated a correlation matrix; to determine statistical variance in the parameters between individual products we used the Student's test; in all the calculations, a level of significance at $P \leq 0.05$ was set. For statistically significant correlations, we created three-dimensional diagrams with the following regression equation:

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_i X_2 + \sum_{i>j}^{k} b_{ij} X_i X_j$$

From the equation presented, Y may be defined as the response for the variables assessed, βo, βi, βi,i,βij are the equation parameter estimates (βo a constant, βi a parameter estimate for linear terms,βi,i an estimate for quadratic terms and

βij an estimate for interactive terms). i and j are the levels of the factor with k being the number of factors assessed (8).

RESULTS AND DISCUSSION

Table 1 shows the results of sensory evaluation (0 = lowest rating, 100 = highest rating – the two are the values read from the graphic scale converted to percents). No difference ($P \leq 0.05$) in the average rating of the colour, matrix, and texture parameters was found between producers. Producer A attained a lower rating in the aroma and taste whereas producer B showed a lower rating of consistency and the producer C differed in the rating of appearance on the cut surface, which was lower than with the other two.

Table 1. Results of sensory analysis, mean values with standard deviation, with maximum and minimum in parentheses

	Producer A	Producer B	Producer C
Appearanceon the cut surface	78.9 ± 6.9[a] (62; 87)	77.5 ± 4.8[a] (68; 82)	63.9 ± 9.8[b] (48; 80)
Colour	76.0 ± 7.4[a] (60; 85)	75.8 ± 7.8[a] (57; 85)	68.9 ± 10.4[a] (50; 83)
Matrix	76.0 ± 6.3[a] (64; 86)	76.6 ± 4.7[a] (67; 83)	74.8 ± 4.6[a] (67; 83
Aroma	69.9 ± 5.9[a] (60; 79)	79.3 ± 6.9[b] (63; 88)	78.2 ± 6.1[b] (64; 85)
Consistency	72.5 ± 8.6[a] (61; 86)	84.2 ± 3.4[b] (78; 89)	78.1 ± 6.3[a] (63; 87)
Texture	81.3 ± 3.6[a] (74; 86)	80.1 ± 6.1[a] (67; 85)	79.6 ± 6.9[a] (67; 87)
Taste	61.7 ± 8.9[a] (48; 73)	76.4 ± 6.3[b] (67; 88)	73.1 ± 13.0[b] (44; 85)

[*] — Within a row, different letters denote significant differences ($P \leq 0.05$) between producers

Generally, we can say that, with regard to sensory evaluation, there is little difference between the individual producers.

When characterizing a product typical of a specific country or region, authors use, among others, chemical composition (10, 4). In Morcilla de Burgos (10), a high variability in the content of fats 28.65 ± 4.92, proteins 13.09 ± 2.3 and ash 4.26 ± 0.49 was found, which the authors explained by variability in the place of origin. In contrast, a small range of results was found in the typical Sicilian salami (5): moisture content 26.8 ± 0.74, proteins 31.5 ± 0.89, fat 33.9 ± 1.17 and NaCl 5.9 ± 0.19. Both authors (10, 5) took their samples just once, in contrast to the present study, where samples were taken over a thirteen-week period.

From Table 2 we can see only little variance in the average chemical composition of the product between individual producers. There is a statistically significant difference ($P \leq 0.05$) in the NaCl and ash content with producer C with respect to the other two producers, and a difference ($P \leq 0.05$) in the fat content with the producer A.

Table 2. Results of physicochemical and textural analysis, mean values with standard deviation, with maximum and minimum in parentheses

	Producer A	Producer B	Producer C
NaCl (%)	2.85 ± 0.36[a] (2.39; 3.37)	2.89 ± 0.28[a] (2.52; 3.38)	3.19 ± 0.25[b] (2.70; 3.57)
Fat (%)	41.63 ± 4.09[a] (36.65; 50.00)	38.08 ± 3.78[b] (29.53; 43.49)	38.59 ± 5.17[b] (25.22; 43.71)
Dry matter (%)	65.08 ± 3.23[a] (60.56; 70.16)	64.52 ± 8.29[a] (54.57; 87.80)	65.08 ± 5.29[a] (55.27; 70.58)
Ash (%)	3.44 ± 0.35[a] (2.97; 4.19)	3.75 ± 0.42[a] (3.15; 4.62)	4.04 ± 0.23[b] (3.62; 4.40)
Collagen (%)	2.54 ± 0.29[a] (2.25; 3.04)	3.01 ± 0.79[a] (2.04; 4.25)	2.87 ± 0.76[a] (1.72; 4.26)
PMP (%)	13.33 ± 1.85[a] (10.21; 15.77)	13.32 ± 2.64[a] (8.49; 17.31)	14.55 ± 2.09[a] (10.72; 17.92)
Shear force (N)	22.2 ± 8.1[a] (14.0; 42.0)	23.3 ± 7.6[a] (14.0; 38.0)	22.3 ± 7.3[a] (15.0; 40.0)

PMP — pure muscle proteins; [*] — Within a row, different letters denote significant differences ($P \leq 0.05$) between producers

No difference in shear force was found between the producers. The products have equal hardness, which only varies with different production batches. A relatively big difference between the minimum and maximum measured value was caused by sample inhomogeneity (the presence of isolated collagen particles).

We generated a correlation matrix (Table 3), in which we can see that parameters that show covariance. Positive and negative coefficients indicate direct and inverse proportion, respectively. The more the coefficient approximates +1 or –1, the more linear is the correlation. If the value approximates zero, the correlation is fully nonlinear. Highlighted coefficients are of statistical significance ($P \leq 0.05$).

The appearance on the cut surface and the content of ash show a negative correlation coefficient indicating an inverse correlation between the parameters. In contrast, the appearance on the cut surface (evaluators mostly focused their attention on particle size) and the matrix (presence of air bubbles show positive correlation) (Fig. 1).

Therefore, we can say that when the product is free of air bubbles and, therefore, is well processed, its particle size approaches the legislative standard. The consistency, odour and taste are parameters that are mutually

Table 3. Correlation matrices of the obtained parameters, statistically significant correlation coefficients are highlighted ($P \leq 0.05$)

	NaCl	Fat	Dry matter	Ash	ACS	Colour	Matrix	Odour	Consistency	Texture	Taste	Collagen	PMP	Shear force
NaCl	–	0.25	0.28	0.70	-0.34	-0.21	0.05	0.15	-0.01	-0.03	0.20	0.10	0.22	0.17
Fat	0.25	–	0.44	-0.05	-0.06	0.23	0.19	-0.01	-0.23	-0.03	-0.00	-0.21	0.21	0.20
Dry matter	0.28	0.44	–	0.44	-0.15	0.24	0.14	0.04	-0.04	-0.28	0.10	0.14	0.47	0.10
Ash	0.70	-0.05	0.44	–	-0.54	-0.21	-0.22	0.24	0.11	-0.25	0.20	0.36	0.42	0.12
ACS	-0.34	-0.06	-0.15	-0.54	–	0.35	0.55	-0.05	-0.00	0.31	-0.05	-0.21	-0.32	-0.13
Colour	-0.21	0.23	0.24	-0.21	0.35	–	0.36	0.30	0.20	0.18	0.34	-0.14	0.40	-0.14
Matrix	0.05	0.19	0.14	-0.22	0.55	0.36	–	0.06	0.33	0.26	0.11	-0.20	-0.14	-0.31
Odour	0.15	-0.01	0.04	0.24	-0.05	0.30	0.06	–	0.54	0.22	0.80	0.21	0.39	0.07
Consistency	-0.01	-0.23	-0.04	0.11	-0.00	0.20	0.33	0.54	–	0.23	0.61	0.05	0.20	-0.16
Texture	-0.03	-0.03	-0.28	-0.25	0.31	0.18	0.26	0.22	0.23	–	0.24	-0.39	-0.25	-0.62
Taste	0.20	-0.00	0.10	0.20	-0.05	0.34	0.11	0.80	0.61	0.24	–	0.26	0.45	0.06
Collagen	0.10	-0.21	0.14	0.36	-0.21	-0.14	-0.20	0.21	0.05	-0.39	0.26	–	0.35	0.50
PMP	0.22	0.21	0.47	0.42	-0.32	0.40	-0.14	0.39	0.20	-0.25	0.45	0.35	–	0.31
Shear force	0.17	0.20	0.10	0.12	-0.13	-0.14	-0.31	0.07	-0.16	-0.62	0.06	0.50	0.31	–

PMP – pure muscle proteins, ACS – appearance on the cut surface

linked; low rating for one of the parameters implies low rating for the remaining ones. The parameters are directly proportional and the correlation draws very near to linear (Fig. 2).

Fig. 1. Correlation between matrix, ash content, and appearance on the cut surface

Fig. 2. Correlation between consistency, odour, and taste

An interesting and, as concerns the measured results, very important chemical indicator is the content of pure muscle protein. At the level of significance $P \leq 0.05$, this parameter shows covariance with the other five parameters. These include dry matter, ash (Fig. 3), colour, odour and taste. This is always a direct proportion with the correlation coefficient within the limits from 0.39 to 0.47. Therefore, it is not a linear correlation but certainly, we

can say that pure muscle protein positively influences the colour, odour, and the taste of the product.

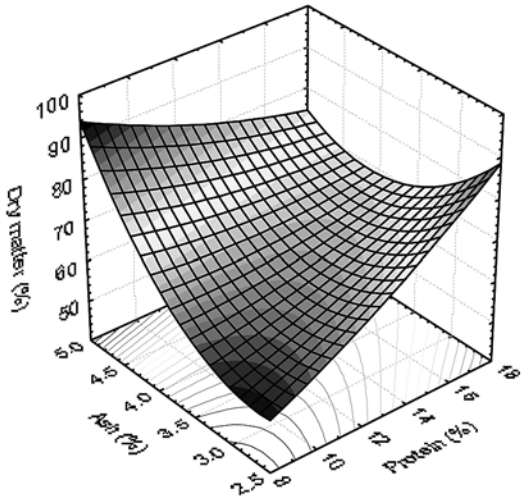

Fig. 3. Correlation between protein, ash, and dry matter

Most interestingly, mutual correlation was found between the parameters of shear force measured instrumentally, texture obtained from sensory evaluation (with an emphasis on the presence of hard particles) and collagen measured chemically.

Fig. 4 indicates that the maximum force required to cut a sample (hardness) increases with an increasing amount of collagen. Conversely, increasing the amount of collagen negatively affects the rating of texture, precisely owing to the amount of hard particles. The correlation between the instrumentally measured texture and the sensory evaluation varies from 0.16 to 0.94 (7); in the present study we obtained the value 0.62 (Table 3). High shear force indicates high hardness of a sample and the presence of stiff collagen particles in it and, consequently, lowers the acceptability of product hardness by the evaluators.

In comminuted scaled sausages, Pietrasik (8) has found out that sausage hardness decreased with increasing content of fat and moisture. Murphy *et al.* (6) have come to a similar conclusion for pork sausages, where shear force decreased with an increasing amount of fat and water. This study, however, offers different results whereby the total amount of fat does not influence either the sensory or textural parameter at a level of significance $P \leq 0.05$, although there are the fat grains that constitute the mosaic of the Vysocina sausage.

Gimeno *et al.* (3) have not found any correlation to exist between the content of proteins and textural parameters, which is consistent with the results of this study. At the level of significance $P \leq 0.05$, no correlation was found to exist between the content of pure muscle proteins and textural parameters measured instrumentally or obtained from sensory evaluation.

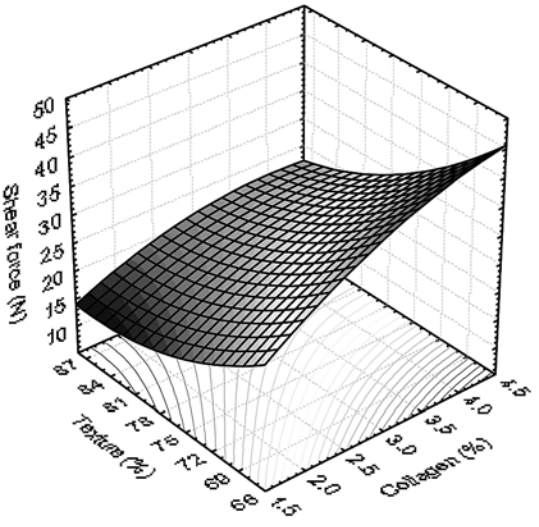

Fig. 4. Correlation between collagen, texture, and shear force

CONCLUSION

The primary aim of this work was to determine whether Vysocina sausages from different producers differ in physico-chemical, sensory and instrumentally measured parameters. Only small variation in the product chemical composition exists between different producers. The composition of the Vysocina sausage, however, varies with different batches from the same producer. No statistically significant difference ($P10 \leq 0.05$) was found between individual producers in the instrumentally measured shear force, which objectively measures product hardness.

The relatively high values of standard deviation of the shear force between individual producers (22.2 ± 8.1 N, 23.3 ± 7.6 N, 22.3 ± 7.3 N) are probably caused by sample inhomogeneity. For consumers, the sensory quality is the most important. Only a minimum difference in the sensory parameters exists between the producers.

The second aim of the work was to determine the mutual correlation between the sensory, instrumentally and individual chemical measured parameters. A correlation between several sensory parameters (see Table 3) was found. The content of fat does not correlate with any of the examined parameters except dry matter. The most important chemical indicator is the content of pure muscle proteins, which correlates with the other five parameters (content of dry matter, ash, colour, odour, and taste).

Correlation between three parameters – texture obtained from sensory evaluation, instrumentally measured shear force indicating product hardness, and chemically determined collagen content – is worth emphasizing. From the shear force and the collagen content, one can

assume how the evaluators will perceive the texture of the product.

Czech legislative sensory requirements for Vysocina according to (11) in all analyzed samples were observed. From the chemical parameters (according to (11)) there was fat content in the limit set in all samples, but there was lower pure muscle protein content in four samples than required.

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THE EFFECT OF DIETARY AMOXYCILLIN ON PORCINE PROLIFERATIVE ENTEROPATHY (PPE) (A short communication)

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ABSTRACT

In a large Croatian growing-finishing pig production unit with a high prevalence of Porcine Proliferative Enteropathy (PPE) growing pigs were feed medicated with amoxycillin. The serological presence of *Lawsonia intracellularis*, clinical signs, mortality and weight gain were evaluated. All pigs revealed positive IFA test results. Amoxycillin treated pigs had significantly lower (P< 0.001) diarrhoea scores, clinical impression scores, and mortality and a higher average daily weight gain compared with untreated animals. The essence of this report is that amoxycillin given shortly after weaning to growing pigs, successfully diminishes acute clinical signs, mortality and improves the performance of pigs.

Key words: antioxidant enzymes; bendiocarb; kidney; spleen liver; thymus

INTRODUCTION

Porcine proliferative enteropathies (PPE) are caused by *Lawsonia intracellularis* (LI) (2). The disease takes four different forms: porcine intestinal adenopathy (PIA) an abnormal proliferation of the cells that line the intestines; necrotic enteritis (NE) where the proliferated cells of the small intestine die and slough off with a gross thickening of the small intestine (hosepipe gut); regional ileitis (RI), inflammation of the terminal part of the small intestine and proliferative haemorrhagic enteropathy (PHE).

In a large Hungarian field study on 15,000 growing-finishing pigs, 29 % of the culled animals had LI infection (1). Therapeutic methods in Eastern Europe against PPE caused losses include continuous or pulse in feed application antibiotics. There are no published reports on the effectiveness of short-term application of amoxycillin against PPE.

MATERIAL AND METHODS

The present study was conducted in a large Croatian growing-finishing pig production unit. In this unit PPE caused high economic losses during the winter of 2004.

The unit was free of classical and African swine fever and *Salmonella choleraesui*s. Pre-trial necropsies of shortly weaned pigs (20–40 days postweaning) revealed the gross pathological signs of acute PPE. Further laboratory analyses were made at Vet-Invest, Zagreb, Croatia: an indirect immunofluorescence antibody (IFA) serum assay and PCR for *Lawsonia intracellularis* (LI) showed positive results. The same necropsied pigs had a low prevalence of beta-haemolytic *Escherichia coli, Actinobacillus (A.) suis, Haemophilus parasuis, Streptococcus suis,* and *Brachyspira hyodysenteriae*. An ELISA test, using a Tween-20 detergent-extracted antigen confirmed low mycoplasmal infection and ELISA for detection bacterial antigen against *A. pleuropneumoniae* serotypes 1, 2, 5, 7 and

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9 showed positive results. No toxigenic *Pasteurella multocida* strains were found in this unit.

The present study was conducted between April and July 2005 in eight replicates on 5,950 randomly selected growing pigs. The pigs were assigned at weaning in each replicate to either:

– amoxycillin feed medication (Group 1: n = 3114, 250 ppm. Amoxycillin trihydrat, Amoxan® 70, UFA, Sursee, Switzerland) during a three-week period, or

– were not treated (Group 2, n = 3202).

The following parameters were evaluated:

Number and percent of LI IFA positive pigs

Ten percent of the pigs in a group (300 pig in each group) were tested for seroprevalence for LI by a serum assay to detect anti-LI IgG antibodies (2).

Diarrhoea score (DS) in a pen (number of days when diarrhoea were observed in a pen and the average number of daily DS on at least one pig in a pen)

1. 0 = no diarrhoea,
2. semi-solid faeces, no blood,
3. watery faeces, without dark or bloody appearance,
4. blood-tinged faeces,
5. profuse diarrhoea with blood or dark tarry faeces.

Clinical impression score in a pen (CIS)

CIS includes the average sum of "attitude score" and "abdominal appearance score" on at least one pig in a pen

Attitude score:

1. normal,
2. depressed,
3. recumbent.

Abdominal appearance score:

1. normal,
2. moderately gaunt,
3. wasting.

Pigs average weight gain during the growing-finishing phase (total pen weight gain divided by animal days in a pen).

Mortality (mortality was defined as the percentage of death in a pen due to PPE).

Statistical analysis

The statistical analysis was performed according to SAS/STAT User's Guide (4th edn. Version 6. Vol. 2, Cary, North Carolina, SAS Inst. Inc. 1989). The experimental design of the study was a randomized block design with the pen as the experimental unit. Pigs were selected at random (computer-generated list within blocks of similar age) and blocked by weight to reduce the impact of weight as a potential confounding factor. Treatment efficacy was assessed by measures of pen mortality, diarrhoea, clinical impression and performance.

The final weight variable was regressed on birth-weight and sex retaining variables with a P-value of 0.05 for the multivariate model in a backward elimination process. To control for the effect of birth-weight on weight gain, birth-weight was included in the final regression analysis. Variables based on pen percentages were transformed using Freeman-Tukey arcsin to satisfy assumptions for the statistical analyses better. Mean-values and the P-values for a one sided *t*-test ($a = 0.05$) of treatment comparisons represent the result of the pooled analysis. A one-factor analysis of variance with the treatment group as the source of variation was performed. Mortality was analysed using Fischer's exact test.

RESULTS AND DISCUSSION

All pigs revealed a positive IFA test results. The amoxycillin treated pigs had significantly lower ($P < 0.001$) diarrhoea scores (during 9.4 ± 1.6 days with av. 1.5 ± 0.4 DS scores vs. 45.6 ± 4.9 days with av. 3.0 ± 1.3 DS scores), clinical impression scores (during 2.0 ± 0.2 days with av. 1.0 ± 0.1 CIS scores vs. 47.7 ± 5.1 days with av. 1.2 ± 0.7 CIS scores), and mortality (1.0 ± 0.3 % vs. 14.4 ± 3.1 %) compared with the untreated animals. Average daily weight gain was significantly ($P < 0.001$) higher in amoxycillin treated pigs compared with the untreated animals (809 ± 74 g SD vs. 601 ± 109 g SD).

Our hypothesis was that amoxycillin medication of pigs would prevent the outbreak of PPE and improve piglet performance. As the results show, a short-term application amoxycillin exerted a beneficial effect on all of the evaluated parameters.

The authors recommend amoxycillin in the prevention and treatment of PPE.

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CHRONICLE

Prof. DVM. František Hrudka, DSc the first Editor-in Chief of FOLIA VETERINARIA

In the past year, Prof. Dr. F. Hrudka, DSc., the founder of Slovak veterinary histology and cytology, a former member of the Scientific Board and academic official of the Veterinary College in Košice (presently UVM), and a former professor of the Veterinary Faculty in Saskatoon, Canada, celebrated his important life jubilee, 80th birtday anniversary. His name is connected with the founding of publication of FOLIA VETERINARIA as the Editor-in-Chief from 1956 to 1958.

Prof. Hrudka was born on 28th October, 1920, in Hôrka, a district of Nové Mesto nad Váhom in western Slovakia. In 1940 he entered the Veterinary College in Vienna, where most of the students from Slovakia, interested in veterinary medicine, had the opportunity to study. During World War II the Veterinary College in Brno, the only establishment of its kind in the former Czechoslovakia, was closed by the decision of German authorities together with all the other colleges in the Czech and Moravian Protectorate. In the second half of 1944, F. Hrudka obtained Diploma in veterinary medicine (DVM.) in Vienna. In the years 1945—1946 he successfully completed his study at the Institute of Exotic Veterinary Medicine in Paris-Alfort.

Upon returning to Slovakia, in the years 1946—1950, he was employed at the Veterinary section of the Mandatory for Agriculture in Bratislava. In 1947 he was appointed head of the section for artificial insemination of mares (focused on the prevention of dourine which spread throughout Europe as a result of the war). In 1950, the rector of the newly established Veterinary College in Košice (in 1949, as the secont establishment of its kind after Veterinary College in Brno) apppointed him the head of the Department of Histology and the Embryology. He became successfully involved in the pedagogical, organizational and research activities of the new college. These activities included also his participation in the publication of FOLIA VETERINARIA, the official scientific journal of the Veterinary College in Košice, as the Editor-in-Chief (in 1956—1958 when the first two volumes were issued).

While being on the staff of the Veterinary University in Košice, Dr. Hrudka occupied successfully various posts and passed through several pedagogical-research stages up to the defence of his doctoral thesis (DSc) at the Medical Faculty of Charles' University in Prague. He became a well known specialist in the field of veterinary histology and embryology not only at home but also abroad and participated actively in many scientific events focused on the field of reproduction of farm animals.

In 1967, Prof. Hrudka was invited for a one year study stay to Canada where, in September 1968, he joined the Veterinary Faculty of the University in Saskatoon, in the south-west part of Canada. His engagement at this institution, prolonged due to political events in the Czechoslovak Republic, was as successful as that in Košice. In 1968—1988, he became a lecturer in histology at the Faculty for Postgraduate Education and Research and led courses on spermiology and cytochemistry (for human and veterinary doctors and biologists). Gradually, he became well known worldwide, through his active participation in world veterinary congresses (from 1967 to 1987). After 38 years of an academic career Prof. Hrudka retired in 1988, i.e. at the age of 68.

Prof. Hrudka returned to Slovakia in the first year of its existence as an independent state, in April 1993, when he was given back his diploma of university professor (at the session of the Scientific Board of UVM in Košice, within extra-judicial rehabilitation). He repeatedly returned bac (in 1998 and 1999) to his home region – central Považie. This is a region associated with other important personalities of Slovak veterinary medicine. Many years ago this was the birthplace of Prof. Pavel Adámi (1736—1814) and Prof. Dr. J. Marek 1868—1952), both of them the members of the staff of Veterinary College in Budapest, and later on also of Prof. Karol Fried (1922—1998), the founder of a Museum of History of Veterinary Medicine in Slovakia, who in his articles acquainted the veterinary community with the above mentioned personalities (including Prof. Hutyra).

The members of Editorial board of FOLIA VETERINARIA join many other will-wishers in wishing good health and well being in the years to Prof. F. Hrudka, the first Editor-in-Chief of the half century old scientific journal of the University of Veterinary Medicine in Košice.

The Editorial Board of
FOLIA VETERINARIA

DVM Michal Breza, PhD
the first Executive Editor-in Chief of FOLIA VETERINARIA

On September 25, 2005. DVM Michal Breza, CSc, was celebrating his 80th birthday. He was born in Trebišov, graduated from the grammar school in Michalovce and continued his studies at the Veterinary University in Brno.

After graduation from the University, Dr. Breza was appointed to the post of a lecturer at the Institute of Veterinary Chemistry by the first rector – at that time – Veterinary College in Košice, Prof. Hovorka. Chemistry was, however, only a temporary stop on his journey towards veterinary parasitology. He joined the Institute of Parasitology, headed by Prof. Hovorka, shortly after the summer term in 1952.

The first half of the 50's in Czechoslovakia were rather challenging times, trying to eradicate parasitic diseases of farm animals. For a young parasitologist it was an opportunity to fully expound his theoretical and practical abilities and skills. Thus gaining experience in diagnostics and epizootiology of cattle dictyocaulosis, ruminants' fasciolosis, enterostrongyloses and other helminthoses. During his PhD study he was engaged in epizootiology of swine metastrongylosis, at that time a frequent disease in Slovakia. He developed a new flotation technique, a new preserving agent for pneumohelminths, and presented new approaches in how to recognise the earthworms' role as an intermediate host, and allotriophagy as a supplement way of nutrition.

Dr. Breza, apart from being an assistant lecturer, has often lectured on Parasitology. After implementing the subject of Veterinary Zoology, he was fully in charge of lecturing and examining, trying to give his students a solid understanding in parasitology and other scientific disciplines. After decade at the Department of Parasitology at the Veterinary College in Košice, Dr. Breza at the end of 1962 became the intern research worker of the Helminthological institute of SAS in Košice, serving as the head of the Department of the Host-Helminth relations till 1988. In 1978, he was also appointed as an executive editor of an international journal *Helminthologia* (until 1992). This position has enabled him to utilize experience from previous editorial activities at the Veterinary College in Košice (*Veterinary Journal, Folia Veterinaria*). He also participated in the organising of many international symposia of the Helminthological Institute of SAS.

Among his numerous activities worth mentioning are his post of supervisor and lecturer for doctors of veterinary medicine and laboratory technicians. He also participated as a co-author in the publushing of all three – so far publisched – *Vademecum of Veterinarians* and preparaton and reviewing of *Veterinary Laboratory Techniques* and *Directives on Veterinary Service*, published by the State Veterinary Administration in the former Czechoslovak Republic.

He is also the author of many chapters in several monographs and handbooks, relating to parasitic diseases of domestic and free-living animals and the author of numerous scientific and technical papers in periodicals.

Dr. Breza was also engaged in the education of young research workers (intern and extern), being the supervisor of more than 30 diploma and CSc. – PhD thesis. He has always been strict, consistent, though a fair reviewer of the presented work, often offering a helping and constructive hand. He approached the reviews for various veterinary and other institutions, and editorial boards of scientific journals with similar zeal.

Since 1994, Dr. Breza has been in retirement, but his temperament and enthusiasms have not allowed him to sit about idly. He actively participates in the preparation of *Folia Veterinaria*, published by the University of Veterinary Medicine and until recently he had served as the executive editor of *Slovak Veterinary Journal*. Besides that, he is always willing to give advice to his younger colleagues from the *University* or the *Parasitological Institute of SAS*.

Having a wide range of hobbies and interest, among them gardening, he is also a talented poet, translating from German poems on zoological and parasitological subject matter from the collection *Zoologica Poetica* and publishing them in the *Veterinary reporter* (he is a long-time member of the Editorial Board).

On behalf of his co-workers we wish Dr. Breza in the future years many and continued interest in his work and well being in his personal lief.

Best wishes!

The Editorial Board of
FOLIA VETERINARIA