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FOLIA VETERINARIA, 47, 2, 2003

CONTENTS

FOLIA VETERINARIA, 47, 2: 57—59, 2003

A PRELIMINARY INVESTIGATION INTO SOME ASPECTS OF THE CRANIOFACIAL INDICES OF THE RED SOKOTO (MARADI) GOAT IN NIGERIA

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ABSTRACT

A preliminary investigation of some craniofacial indices of the head of the Red Sokoto goats in Nigeria revealed that the mean weight of the head was 1.04 ± 0.15 kg, the mean palpebral fissure lengths were 2.51 ± 0.17 cm and 2.50 ± 0.19 cm for right and left respectively. The mean distance between the medial canthi of the eyes was 10.43 ± 0.37 cm. These values were higher in animals above fifteen kilograms compared to animals below this body weight. They were also higher in females compared to males. The reverse was however the case for the value of the ratio of weight of brain to weight of head (WOB/WOH) and weight of head to animal body weight (WOH/WOA). These findings were discussed in relation to their significance as baseline tools in research in bio-engineering, genetics, adaptative evolutionary studies, comparative and regional anatomy and as controls in assessing congenital defects and organ pathologies in the head of Red Sokoto goats.

Key words: craniofacial indices; Red Sokoto goats

INTRODUCTION

The head is a very important region for animals. It is the location of such vital organs as the brain, eyes, nose, tongue, ear and mouth. Indeed the health of an animal can be deduced from the functional state of any of these organs. For instance the colour of mucous membranes of the eye and mouth, the degree of wetness of the muzzle are important clinical parameters in assessing the health status of an animal.

The regional anatomy of the head is therefore very important. An important component of the anatomy of any region is the spatial relationships of the organs found in that region. Such studies have been of great value in the study of the interactions between the genetic endowments of an animal and its environment (4, 6, 13).

It was therefore thought necessary to map out the regional anatomy in the head of the Red Sokoto (Maradi) goat for both scientific and clinical purposes. The Red Sokoto goat is an important greed of goats in Northern Nigeria. They are usually slaughtered for meat and their skin commands a high premium in the leather industry (10, 7). Indeed they are an important foreign exchange earner for the country. Aside from this information, very little else is known about the pecularities of the anatomy of this goat breed

The aim of this work was to study the craniofacial indices of the Red Sokoto goat in a continuing effort to provide baseline information on the anatomy of specific breeds of animals in Nigeria.

MATERIALS AND METHOD

Fifteen Red Sokoto goat heads were purchased from the Bodija meat market in Ibadan, South Western Nigeria. The heads were cut at the occipito-atlantal joint, taken to the laboratory and weighed using a five goat spring scale® and measurement of different craniofacial dimensions were done using a Stanley power lock tape®. The heads were frozen and the brains were harvested and weighed using a microwa swiss® machine.

The accruing data were compared using Student's *t*-test at a 5 % level of significance. The animals were separated according to sex and according to a weight barrier of fifteen kilograms. The data generated in this study is expressed on Table 1.

RESULTS

The mean weight of all the goat heads was 1.04 ± 0.15 kg while that of the brain was 85.27 ± 10.02 g. The mean distance between the medial canthi was 10.43 ± 0.37 cm. The medial to lateral canthi of the right eyelid of animals < fifteen kilograms was significantly longer ($p < 0.05$) than that of animals > fifteen kilograms. The animals > fifteen kilograms had significantly heavier brains ($p > 0.05$) compared to animals below that weight mark.

Animals above fifteen kilograms had significantly longer distances ($p < 0.05$) between the medial canthi, the medial to lateral canthus (left and right). MIPI and MIP II compared to animals below fifteen kilograms. The same was true for females in comparison with males.

The females however had significantly lower WOB/WOH and WOH/WOA ratios ($p < 0.05$) compared to males and so did the animals above fifteen kilograms in comparison with those below that weight mark.

DISCUSSION

The mean weight of the head of the female was significantly higher ($p < 0.05$) than that of males. A relatively heavier head muscle mass, cranial bones and brain could have contributed to this. Donaldson (3) had reported that female rats had significantly higher spinal cord weights compared to male rats. There was no significant difference ($p > 0.05$) in the mean distance of the medial to lateral canthus between the right and left eyelids in both sexes but the male had a slightly longer right eyelid. Sisson and Grossman (12) has observed similar asymmetry between the two halves of bodies of some mammals.

Females had significantly longer heads compared to males as evidenced by their higher MIP I and MIP II values. Females also had more widely spaced out eyes (higher distances between the median canthi). The reason for this and its implication for the visual field of the animal is unknown. Age and size differences may be partly responsible however for it is a common practice for livestock farmers in Nigeria to offer their male goats for sale much earlier than the females which are usually kept back on the farm for breeding purposes (11). At any one time, therefore, there are usually age differences between the sexes. The females are invariably older and heavier.

The mean WOB/WOH obtained in this study was 0.08. This value of 8 % could thus be a base line in determining the size of brain in relation to the head in the live animal. By obtaining the volume of blood flow to the head from the formula of mean arterial blood pressure to the head divided by the vascular resistance to that flow (www.nursing/iowa.edu), the weight of brain to that of the head in the live animal can be better estimated and thus the oxidative metabolism of the goat brain in relation to the head also better assessed. In the metabolism of the perfused brain of the dog, approximately 50 % of the oxygen supplied to the head was used by the brain (8).

Males had higher WOB/WOH than females. This suggests that males have relatively heavier brains in relation to head size and this is due to far larger proportions of non-brain constituents the head of the female such as muscles.

Animals lower than fifteen kilograms had 11.0 and 12.5 % higher values of WOB/WOH and WOH/WOA, respectively, when compared to those above this body mark. This suggests that non-brain components of the head are more rapid or heavier in their growth (during the animals growth) when compared to the brain. It also suggests that other parts of the body have a more rapid, heavier or continuous growth in comparison to that of the head. The growth of limbs are pronounced and produce heavier weights compared to those of the sutures of the skull in early life (5).

Through medial to lateral canthus length was significant more in the right eyelid compared to the left in animals

Table 1. Some craniofacial dimensions of the head of the Red Sokoto goat

Parameters	Body weight < 15 kg (n = 7)	Status > 15 kg (n = 8)	Males (n = 10)	Female n = 5	Total n = 15
Weight of animal WOA (kg)	11.71 ± 0.06	18.00 ± 0.00	13.30 ± 2.24	18.60 ± 2.43	15.07 ± 3.68
Weight of animal head WOH (kg)	0.90 ± 0.16	1.17 ± 0.03	0.98 ± 0.13	1.17 ± 0.13	1.04 ± 0.15
Weight of brain (g)	81.66 ± 7.86	88.43 ± 10.79	85.19 ± 8.21	85.42 ± 9.12	85.27 ± 10.02
Right palpebral tissue length (cm)	2.43 ± 0.03	2.59 ± 0.05	2.44 ± 0.12	266 ± 0.14	2.50 ± 0.17
Left palpebral tissue length (cm)	2.40 ± 0.11	2.59 ± 0.05	2.42 ± 0.15	2.66 ± 0.09	2.50 ± 0.17
Distance between medial canthi (cm)	10.19 ± 0.06	10.64 ± 0.31	1037 ± 0.39	10.54 ± 0.21	10.43 ± 0.37
MIP I (cm)	12.16 ± 0.05	13.95 ± 0.01	12.54 ± 0.94	14.26 ± 0.03	13.11 ± 1.40
MIP II (cm)	22.90 ± 0.00	24.94 ± 0.05	23.59 ± 1.55	24.78 ± 0.38	23.99 ± 1.40
WOB/WOH	0.09 ± 0.05	0.08 ± 0.05	0.09 ± 0.01	0.07 ± 0.01	0.08 ± 0.03
WOH/WOA	0.08 ± 0.07	0.07 ± 0.06	0.08 ± 0.08	0.06 ± 0.00	0.07 ± 0.00

MIP I — Frontal intercornual prominence to the muzzle
MIP II — External occipital protuberance to the muzzle
The results were expressed as means ± SD

below fifteen kilograms there was no difference in animals above this weight mark. This could mean that the asymmetry seen in the palpebral fissure is age-legs and be balanced as the animals increase in size or probably as they grow older and increase in weight. Cats have been known to have one of the eyelids more functional and active in the early days of life but both function at par as the animal gets older (1). This functional interplay could perhaps have morphological growth implications.

With parameters in this study significantly higher in animals above fifteen kilograms compared to those below the weight mark, absolute sex differences could not be established since the females in this study weighed more than the males.

The results obtained in this study could act as baseline data in bio-engineering (9), in genetics, adaptative evolutionary studies (4), in comparative anatomy and regional anatomical studies of the goat head (5) and as controls in assessing congenital defects and organ pathologies (2) that may affect the head of the Red Sokoto goat.

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REFERENCES

1. Blakemore, C., Cummings, R. M., 1975: Eye-Opening in Kittens. *Vision Res.,* 155—1417,

2. Cruzz, A., A., Coelho, R. P., Baccega, A., Lucchezi, M. C., Sourza, A. D., Ruiz, E. E., 1998: Computerized bi-dimensional analysis of the palpebral crack. *Opthalmology,* 105, 913—918.

3. Donaldson, H. H., 1909: On the relation of the body length to the body weight and to the weight of brain and of the spinal cord in albino rats *(Mus norvegicus* var. *albus). J. Comp. Neurol.* 155—157.

4. Dunlop, A. A., 1963: Interaction between heredity and environment in the Australia Merion strain X: Location-interaction in body traits and reproduction performance. *Australian J. Agric. Res.,* 14, 690—703

5. Dyce, K., M., Sack., W. O., Wensing, C. J. G., 1996: *Textbook of Veterinary Anatomy* (3rd edn.). W. B. Saunders Company, Philadelphia.

6. Hall, S. J. G., Russel, A. J., F., Hellden, H., 1996: Goat fibres of Nigeria sheep and goats: A preliminary characteristic. *Small Ruminant Research,* 22, 169—175.

7. Hall, S. J. G., 1991: Body dimensions of Nigerian cattle, sheep and goats. *J. Anim. Production*, 53, 61—67.

8. Handley, C. A., Sweeny, H. M., Scherman, Q., Severance, R., 1943: Metabolism of the perfused dog's brain. *Am. J. Physiol*., 140, 190—196.

9. Nemoto, T., Saito, T., Kasuya, E., Honda, Y., Nakamura, M., Kobayashi, T., Chen, W., Tamada, I., Kusunose, R., Kari, M., 2000: Development of an eye camera for measuring eyelid and eyeball movements in domestic animals. *JAR Q,* 34, 271—277.

10. Okali, C., Upton, M., 1984: The potential for increased small ruminant production in South-West Nigeria. *Proceedings of the Workshop on Small Ruminant Production System in Humid Zone of West Africa Held in Ibadan, Nigeria*, 23—26 January, 1984.

11. Onwuka, S. K., Yahaya, M., Furo, P. S., 2001: A survey of the slaughter pattern of small ruminants at the Yola modern abattoir (1987—1996). *Afr. J. Biomed. Res.,* 73—77.

12. Sisson, S., Grossman, 1975: *The Anatomy of the Domestic Animals.* **Getty, R.** (ed.) (5th edition). WB Saunders Company, Philadelphia.

13. Salako, A. E., 2002: *Phenotypic Variations Among the West African Dwarf and Yankasa Sheep of the Humid South-West Nigeria.* PhD. Thesis, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

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THE POSSIBILITY OF ENDOPARASITOSIS DAMPING BY ECOLOGICALLY CONSIDERED METHODS IN HORSES

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ABSTRACT

The efficiency of the Ivomec and the homeopathic preparation États Vermineux (company Boiron), on Strongyli-dae (Strongylinae and Cyathostominae) and Ascaridae were compared in two groups of six mares and their foals. Control was carried out by the coprological analysis of faeces. We can state that the anthelmintic effect of both preparations was all but identical in their persistence and in their efficiency. It is more advantageous to use this homeopathic preparation if we consider the toll on the animal body and the environment in terms of residues and economy of treatment.

Key words: allopathic treatment; efficiency; endoparasites; homeopathy; horses

INTRODUCTION

Parasitic infections usually occur in herds of horses under normal conditions. Their occurrence increases particularly during grazing. The negative effect of endoparasites is manifested in an aggravated health condition, especially in the lower efficiency of contaminated (affected) individuals.

Insufficient animal hygiene is a frequent cause of endoparasitoses in horse breeding. Parasitic diseases are impossible to stamp out completely, but it is necessary to contain parasitic infection at a minimal level. Treatment methods, which are used to inhibit helminthiases at present, are based on chemotherapy. The most frequently used are various wide-spectral anthelmintics (6). Their effectiveness is similar, but the price per dosage is different (7).

The development of resistance and occurrence of residues in the organism, products and also in excrements indicate the negative impact of anthelmintics. Residues from animal faeces and urine can also have a negative effect on soil microflora. Consequently, unconventional methods for inhibition of parasitoses have gained prominence in scientific and practical attention. There are vaccines against helminths, genetic manipulations with parasites and support for the spread of plants with an anthelmintical effect in pastures, but also for an anthelmintic treatment, for instance (6, 5).

Sometimes it is appropriate to use allopathic means together with homeopathic treatment (3). Czech organic farming stipulates (1) that natural and homeopathic drugs have a priority in the treatment of sick animals.

Of course, we cannot indicate the individual components of the homeopathic drugs as agents able to kill the parasites, but as field modifiers of an organism. As such, they support the natural defense processes of the affected organism. They have no contraindications and undesirable secondary effects and it is impossible to administer an overdose with them (4).

The purpose of the experiment was to assess the possibility of using unconventional methods of treating endoparasitoses in horses by a homeopathic preparation, États Vermineux, for verminous conditions and to compare its effectiveness and time of treatment with the commonly used Ivomec.

MATERIALS AND METHODS

A herd of horses in Napajedla (The Czech Republic) was included in the observation. At the beginning, six mares with foals, that is twenty-four in total, were used in the trial and control groups.

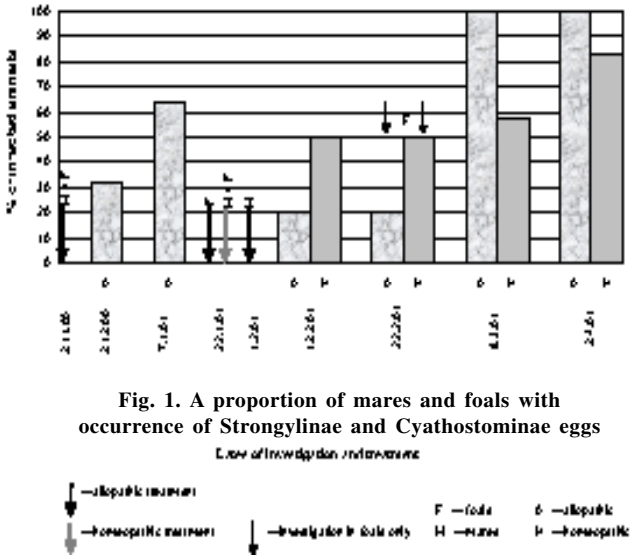

Fig. 1. A proportion of mares and foals with occurrence of Strongylinae and Cyathostominae eggs

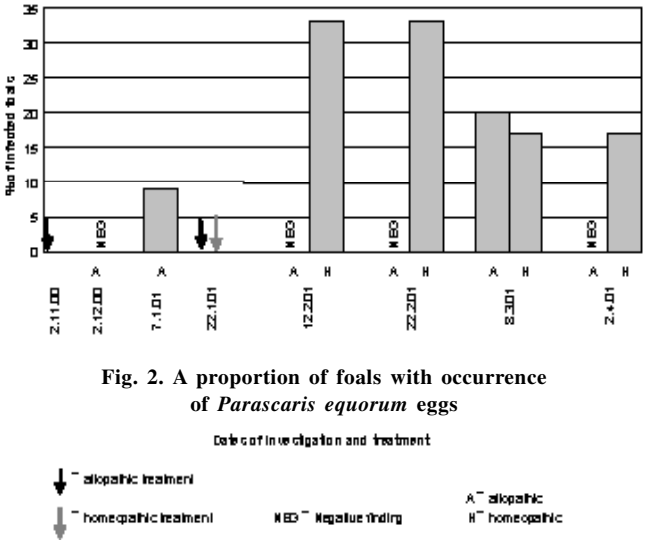

Fig. 2. A proportion of foals with occurrence of *Parascaris equorum* eggs

The trial group was dewormed with the polycomponental homeopathic preparation, États Vermineux, for verminous states (company Boiron, France). The application was administered orally once per day for eight consecutive days. The control group was dewormed with the preparation, Ivomec, on the day when the homeopathic treatment in the trial group ended.

Faeces samples were taken for coprological analyses at the beginning of the application of the homeopathic preparation and on the fourteenth day after the completion of the application of all preparations in both groups. The occurrence of eggs of Strongylidae (strongylidiasis) and Ascaridae (ascaridiasis) by the flotation method (2) for the determination of the intensity of egg elimination was used.

RESULTS AND DISCUSSION

We did not find significant differences in comparing the numbers of eggs of Strongylidae *(Strogylinae* and *Cyathostominae)* in faeces between groups dewormed by Ivomec and those treated by homeopathic preparation PVB. The effectiveness of both these preparations is similar as far as the intensity and time of action are concerned.

Mares and foals are protected against a recurrence of infection for approximately two months. The response of foals is slower in the application of homeopathics than in the application of Ivomec and the occurrence of infection is higher in the trial group than in the control group (Fig. 1). In contrast, the experimental group of mares were dewormed very fast, with a negative discovery of *Strongylidae eggs*. In the mares of the control group, eggs of these nematodes were more frequent and with a higher amount.

The occurrence of *Parascaris equorum* eggs was not found in mares (Fig. 2). In the foals the occurrence was confirmed above all in the group treated homeopathically. The result of the experiment confirmed the validity of arguments by Prasličkа *et al.* (6), that it is necessary to search for new alternative methods of the treatment of parasitoses. These methods should have a similar effectiveness as conventional drugs, however, with a lower stress experienced by the animals treated and without residues. The ideas of Issautier and Calvet (3), Issautier (4) and Marečková (5) that it is suitable to use allopathic preparations together with homeopathic treatment were verified in our trial. The significantly lower costs of this kind of treatment are not negligible (7, 4).

CONCLUSION

On the basis of the results of the coprological examination of faeces of mares and their foals we can conclude that the anthelmintic effectiveness of both preparations employed, Ivomec and PVB, against Strongylidae is comparable as far as the intensity and the time of influence are concerned. Ivomec is better against *Parascaris equorum.* Alternating treatment with both preparations seems to be the most appropriate. Using homeopathic drugs is more advantageous from the view of stress the organism is exposed to and the rise of residues in the environment.

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REFERENCES

1. Anonymous, 2000: Zákon č. 242/2000 Sb., o ekologickém zemědělství a o znění zákona č. 368/1992 Sb., o správních poplatcích, ve znění pozdějších předpisů a Vyhláška Ministerstva zemědělství č. 53/2001 Sb., kterou se provádí zákon č. 242/2000 Sb., o ekologickém zemědělství a o změně zákona č. 368/1992 Sb., o správních poplatcích, ve znění pozdějších předpisů s komentářem. Praha, MZe ČR, 2001 (In Czech), 78 pp.

2. Breza, M., 1957: Some practical knowledge and suggestions for helminth-coprological diagnostics (In Slovak). Miscellany: *Helmintológia.* Liblice, 1957, 57—63.

3. Issautier, N. M., Calvet, H., 1992: *Veterinary Homeopathic Therapy* (In Czech). Publ. House Vodnář, Prague, 428 pp.

4. Issautier, N. M., 1995: *Vademecum of Veterinary Homeopathic Preparations — Series PVB and VETOPHYL* (In Czech). Publ. House Vodnář, 136 pp.

5. Marečková, A., 1998: Experiences in homeopathy in a small animal practice (In Czech). *Veterinářství,* 48, 19.

6. Praslička, J., Letková, V., Lukešová, D., 1999: Alternatives for nematode control in connection with anthelmintic resistance (In Slovak). *Vet. Med.-Czech*, 44, 83—89.

7. Špicera, J., 1998: *The Choice of Useful Anti-parasitary Preparation* (In Czech). Svaz chovatelů koní Kinských, Chlumec nad Cidlinou a Česká zemědělská univerzita v Praze, 29 pp.

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A MORPHOMETRIC ANALYSIS OF THE UTERUS IN THE WOOD MOUSE (*Apodemus sylvaticus*) AND THE YELLOW-NECKED MOUSE (*Apodemus flavicollis*)

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ABSTRACT

In this study a morphometric analysis of the uterus in wood mouse (*Apodemus sylvaticus*) and yellow-necked mouse (*Apodemus flavicollis*) was undertaken. In the uterus of the wood mouse the relative volume of the endometrium is 72.5 ± 11.84 % and of the myometrium 27.5 ± 11.84 %. In the endometrium the relative volume of surface epithelium is 5.7 ± 2.98 %, glandular epithelium 10.3 ± 9.76 %, and the stroma forms 84.0 ± 8.58 %. In the yellow-necked mouse the relative volume of the endometrium is 61.5 ± 7.1 % and of the myometrium 38.5 ± 7.1 %, the stroma forms the highest relative volume (83.5 ± 6.00 %). The relative volume of surface epithelium is 13.7 ± 4.88 %, and glandular epithelium forms 2.8 ± 1.12 %. In the correlation analysis we found the high negative correlation between the relative volume of the surface epithelium and glandular epithelium (0.955). The relative volume of the surface epithelium has a medium negative correlation (0.525) with glandular epithelium. The correlation between the epithelium and stroma was low (0.249). From these results it may be suggested that the structure of the uterus in both species studied is very similar without significant differences.

Key words: morphometry; uterus; wood mouse; yellow-necked mouse

INTRODUCTION

The wood mouse (*Apodemus sylvaticus)* can be found widespread in Europe, from the Iberian peninsula to western Russia, as far north as Iceland, southern Norway and Sweden. In the Mediterranean, it is found on the most of the islands, including Corsica, Sicily and Crete. Peak annual densities in woodland may exceed 50 individuals per ha in heavy mast years (12).

The yellow-necked mouse (Apodemus flavicollis) lives more northerly on mainland Europe than A. sylvaticus, in Finland and Sweden north to 64 °N, east to the Urals. It is absent from western France and much of the Iberian peninsula. The density of the population in mature deciduous woodland usually reaches 60 individuals per ha in Great Britain and over 100 individuals per ha in eastern Europe (13).

Reproductive organs are not important for the life of an animal, but they are important for the reproduction of species and are among the most active organs. Morphometric analyses have been reported mainly in farm animals (1, 3, 6, 7, 14) and only few describe the structure in wild animals (10, 11).

The breeding season in small mammals in North Europe begins in April, but when the winters are mild and spring starts earlier, breeding can also begin in March. In most populations through all the year the last delivery occurs in early September (6, 13). In our conditions the breeding season began earlier (February) and takes longer – till November (2, 4, 5, 6).

The purpose of this study was to quantify the microscopic structure of the uterus in the wood mouse (*A. sylvaticus*) and the yellow-necked mouse (*A. flavicollis*) and to compare this data with previous findings.

MATERIAL AND METHODS

The samples were taken from wood mice (n = 15) and yellow-necked mice (n = 15), which were caught in the surroundings of Mochovce, west Slovakia at the middle of May 1997. All animals were adult, in good condition. The average weight of the wood mice was 27.2 g and the average length was 93.0 mm. The yellow-necked mice averaged 37.1 g in weight and 99.3 mm in length.

After collecting the samples (middle part of uterus), they were fixed in 10 % formol. After fixation the samples were dehydrated in a graded series of ethanol (70, 80, 90, and 100 %), saturated in benzene, benzene-paraffin and embedded in paraffin. Blocks of samples were then sectioned on a microtome onto 10 μm thick sections, which were stained with heamatoxylin and eosin (18). From microphotographs (Docuval, Carl Zeiss Jena) based on micromorphological criteria (9, 16, 19) the quantitative values of uterine structure were evaluated with respect to each sample.

In the uterus tissue the qualitative microscopic structure and the relative volume (%) of the endometrium, myometrium, as well as of the surface epithelium, glandular epithelium and stroma in the endometrium were investigated (at magnification 200×).

To compare the results the analysis of variance as well as the Student's *t*-test and correlations were applied (15).

RESULTS AND DISCUSSION

The uterus is a muscular organ and receives the right and left fallopian tubes. It is lined by columnar surface epithelium, which forms glandular epithelium (Figs. 1, 2).

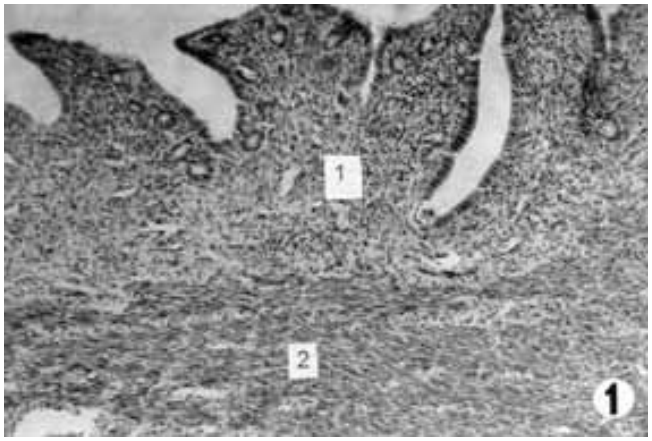

Fig. 1. The uterus wall in wood mice is formed by two layers — endometrium (1) and myometrium (2) (H-E, 100×)

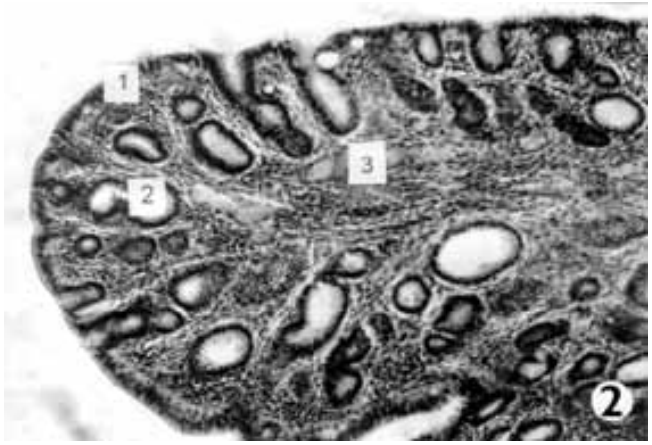

Fig. 2. Detail of endometrium in yellow-necked mice with columnar epithelium (1) forming uterine gland (2). The highest relative volume forms connective tissue — stroma (3) (H-E, 200×)

Both the wood mouse and the yellow-necked mouse have a uterus duplex, i.e. the uterus has two bodies and two cervices.

In the uterus of the wood mouse the relative volume of the endometrium is 72.5 % and of the myometrium 27.5 %. In the endometrium the relative volume of the surface epithelium is 5.7 %, glandular epithelium 10.3 %, and the stroma forms 84.0 % (Table 1).

Table 1. Relative volume (%) of the structures in the uterus of the wood mouse and the yellow-necked mouse

Parameter	The wood mouse		The yellow-necked mouse	
	$\bar{x}$	s	$\bar{x}$	s
Endometrium	72.5	11.84	61.5	7.1
Myometrium	27.5	11.84	38.5	7.1
Surface epithelium	5.7	2.98	13.7	4.88
Glandular epithelium	10.3	9.76	2.8	1.12
Stroma	84.0	8.58	83.5	6.00

In the yellow-necked mouse the relative volume of the endometrium is 61.5 % and of myometrium 38.5 %. Stroma has the highest relative volume (83.5 %). The relative volume of the surface epithelium is 13.7 % and glandular epithelium forms 2.8 %.

In the correlation analysis we found high negative correlation between the relative volume of the surface epithelium and glandular epithelium (0.955). The relative volume of the surface epithelium has a medium negative correlation (0.525) with the glandular epithelium. The correlation between the epithelium and stroma was low (0.249).

In comparison with this species there is a higher relative volume of the myometrium in rabbits (51.27 %) and a lower relative volume of the endometrium in rabbits (8). The surface eptihelium forms 5.9 % in rabbits, glandular epithelium 5.8 % and stroma 88.3 %. Also in the hare it has been reported that the relative volume of the endometrium is 22.86—43.05 % and that of the myometrium 56.95—77.14 % according to the season (11). Fine analysis showed that the surface epithelium forms 3.06—9.45 %, glandular epithelium 8.55—35.40 % and stroma 61.32—88.38 % according to the season. We can report that the data are similar to the species described in this report.

The relative volume of the surface epithelium in cows is 2.3—3.7 %, glandular epithelium 18.2—22.5 %, and the stroma forms 75.2—79.1 % (17). It has been also reported that the changes in the structure and relative volume of uterine cells are not significant. The differences in the relative volume are 1.5 %.

We have to state that in this study we have provided a morphometric analysis of two wild animal species (wood mouse, yellow-necked mouse). There is very little data about the reproduction of these animals, and the stud-

ies describing microscopic structures and morphometric analyses are absent.

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REFERENCES

1. Cigánková, V., Krajničáková, M., Kokardová, M., Tomajková, E., 1996: Morphological changes in the ewe uterine tube (oviduct) epithelium during puerperium. *Vet. Med.- Czech*, 41, 339—346.

2. Clarke, J. R., 1985: The reproductive biology of the bank vole (*Clethrionomys glareolus*) and wood mouse (*Apodemus sylvaticus*). *Symp. Zool. Soc. London*, 55, 33—59.

3. Danko, J., 1997: Ovarian mass, size and number of follicles in postparturient ewes. *Acta Vet. Brno*, 66, 71—74.

4. Flowerdew, J. R., 1985: The population dynamics of wood mice and yellow-necked mice. *Symp. Zool. Soc. London*, 55, 315—338.

5. Gray, C. A., Bartol, F. F., Tarleton, B. J., 2001: Developmental biology of uterine glands. *Biol. Reprod.,* 65, 1311—1323.

6. Hurka, L., Němec, F., 1985: Population Analyse der Art *Apodemus sylvaticus* (*MAMMALIA*: *Muridae*) aus den Gebiet des westlischen Teils der Tschechoslowakei mit Bemerkungen über ihre Siphonapterofauna. *Fol. Mus. Rer. Nat. Bohemiae occidentalis*, Zoologica, 20, 1—34.

7. Kaikusalo, A., 1972: Population turnover and wintering of the bank vole, *Clethrionomys glareolus* (Schreb.) in southern and central Finland. *Ann. Zool. Fennici*, 9, 219—224.

8. Krajničáková, M., Bekeová, E, Lenhart, L., Cigánková, V., Valocký, I., Maraček, I., 1999: Microscopic analysis of the uterine epithelium in postparturient ewes. *Acta Vet. Brno*, 68, 9—12.

9. Massányi, P., Uhrín, V., 1996: Morphometric parameters of ovary, oviduct and uterus in rabbits (*Oryctolagus cuniculus* f. *domestica*). *Acta Zootechnica Nitra*, 52, 89—96.

10. Massányi, P., Uhrín, V., 1997: Histological changes in the uterus of rabbits after an administration of cadmium. *J. Environ. Sci. Health*, A 32, 1459—1466.

11. Massányi, P., Slamečka, J., Lukáč, N., Jurčík, R., 2000: Seasonal variations in the morphometric analysis of the ovary and uterus and in progesterone and 17ß-oestradiol production in the brown hare (*Lepus europaeus*). *J. Anim. Feed Sci.*, 9, 697—708.

12. Montgomery, W. I., 1999a: *Apodemus sylvaticus*. In **Mitchell-Jones, A. J., Amori, G., Bogdanowicz, W., Krystufek, B., Reijnders, P. H. J., Spitzenberger, F., Stubbe, M., Thirssen, J. B. M., Vohralik, V., Zima, J., 1999:** *Atlas of European Mammals*. The Academic Press, London, 274—275.

13. Montgomery, W. I., 1999b: *Apodemus flavicollis*. In **Mitchell-Jones, A. J., Amori, G., Bogdanowicz, W., Krystufek, B., Reijnders, P. H. J., Spitzenberger, F., Stubbe, M., Thirssen, J. B. M., Vohralik, V., Zima, J., 1999:** *Atlas of European Mammals*. The Academic Press, London, 270—271.

14. Pokki, J., 1981: Distribution, demography and dispersal of the field vole, *Microtus agrestis* (L.) in the Tvarmainne archipelago, Finland. *Acta Zoologica Fennica*, 164, 1—48.

15. Rockwell, L. C., Pillai S., Olson C. E., 2002: Inhibition of vascular endothelial growth factor/vascular permeability factor action blocks estrogen-induced uterine edema and implantation in rodents. *Biol. Reprod.,* 67, 1804—1810.

16. SAS, 1998: *SAS/STAT User's Manual Guide* (version 6th edition). SAS Institute, Cary, NC, USA.

17. Uhrín, V., 1992: *Functional Morphology of the Oviduct and Uterine Epithelium in Cows* (In Slovak). Slovak Academic Press, Bratislava, 172 pp.

18. Vacek, Z., 1974: *Histology and Histological Technics* (In Slovak). Osveta, Martin, 388 pp.

19. Weibel, E. R., Kistler, G. S., Scherle, W. F., 1966: Practical sterological methods for morphometric cytology. *J. Cell Biol.*, 30, 33—38.

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PRODUCTION OF WATER VAPOURS AND CARBON DIOXIDE BY BROILERS UNDER EXPERIMENTAL AND OPERATION CONDITIONS

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ABSTRACT

Production of water vapours and carbon dioxide was investigated under experimental and operation conditions in broilers housed on litter up to the age of 6 weeks and the final weight of 2.0 kg.head^{-1} on average. The real values were obtained by calculation on the basis of temperature, relative humidity, and concentration of CO_2 values measured in a known volume of incoming and outgoing air exchanged by an exhaust-type ventilation. The production of water vapours and carbon dioxide in the experiment decreased with increasing weight of broilers from the mean weight of 0.25 kg.head^{-1} always up to the final weight of 1.5—2.0 kg. head^{-1}; the initial values reaching 6.40 g.h^{-1}.kg^{-1} and 1.85 l. h^{-1}.kg^{-1} and the final ones 4.02 g.h^{-1}.kg^{-1} and 1.23 l.h^{-1}.kg^{-1}, resp. In the hall with capacity of 25 000 broilers no decrease in production of the observed noxious components was recorded. The mean production of water vapours for the period of observation was 6.05 g.h^{-1}.kg^{-1} and varied from 7.34 to 4.77 g.h^{-1}.kg^{-1} and the mean production of CO_2 was 1.72 l.h^{-1}.kg^{-1} and varied from 1.84 to 1.53 l.h^{-1}.kg^{-1}.

Key words: bioclimate; broilers; carbon dioxide; water vapours

INTRODUCTION

Under the conditions of intensive fattening of broilers bioclimate becomes a productive even existence factor. Each deviation from the optimum values decreases proportionally the efficiency of rearing both directly and indirectly by decreasing the general resistance of birds. A decisive role in the management of bioclimate belongs to ventilation. The ventilation system as a basic regulator of the microclimate in intensive production halls should ensure by its capacity and arrangement a sufficient exchange of air in accordance with biological load and the external environmental conditions. Under the conditions of central European countries ventilation should comply not only with the requirements of rapidly growing birds but also with those related to the economy of production. The requirements on ventilation in broiler halls depend on production of noxious components. Their production per time unit depends essentially on the weight of broilers, their age, and temperature of the environment. They should be predetermined in a close range for the use in the respective technologies of rearing.

Large number of papers deals with bioclimate in animal houses and the requirements on ventilation (3, 4, 5, 6, 7, 9, 10, 11, 14). However, there is a gap with respect to interconnection of these two aspects and real production of noxious components in animal housings. The knowledge on production of heat and moisture by broilers is based mostly on experiments in climate chambers. The available results are advantageous and accurate under specific conditions, however, in real animal houses completely different conditions may develop (12).

MATERIALS AND METHODS

The experiment was carried out in a closed chamber made from waterproof polyethylene foil located in a separate room of dimensions 8 × 8 × 2.5 m with 2 windows that were constantly opened. The litter consisted of a layer of soft wood shavings. The air volume and floor area per kg of final live weight corresponded to the conditions in broiler fattening halls, i.e. 0.1 m^3.kg^{-2} and 31 kg.m^{-2}, resp. The thermal comfort of chickens was ensured by infra-lamps provided with an anti-reflex layer and the exchange of air by a three-stage manually controlled exhaust ventilation (30, 70 or 120 m^3.h^{-1}). Fresh air was supplied through a slit located close to the floor, designed for

inlet velocity of 0.5 m.s^{-1}. The incoming air was deflected by a board so it entered the chamber at a height of 40 cm. The box was stocked with 36 one day old chickens of ROSS-308 hybrid that were weighed 3 times per week throughout the experiment.

Temperature and relative humidity of the incoming and outgoing air were registered by thermo-hygrographs the function of which was checked daily by means of a digital thermo-hygrometer. The concentration of CO_2 in the outgoing air was measured daily by detection tubes with 0.01 % resolution. The concentration of CO_2 in the incoming air remained constantly at 0.04 % level.

To obtain the real values of production of moisture and carbon dioxide under operation conditions, measurements were carried out during two fattening cycles in a hall housing 25 000 broilers of the same hybrid as that used under experimental conditions. They were housed on wooden shavings and the unidirectional exhaust ventilation was adjusted so that it could be controlled manually. Three thermo-hygrographs were placed next to the ventilation outlets and one outside the building. Digital thermo-hygrometer was again used to check the registering equipment regularly. Carbon dioxide was measured at three locations next to the ventilation outlets using detection tubes.

The production of water vapours was calculated under experimental conditions using mean daily values of temperature and relative humidity and in the intensive fattening hall on the basis of ambulant measurements and means of 6—8 h intervals with maximum amplitude of temperature and relative humidity 4 °C and 10 %, resp., and production of CO_2 from its measured concentrations as follows:

$$Mwu = Vu.\ (x_i - x_e).\rho_i$$

$$Vcu = Vu.\ (Cci - 0.04 \text{ or } 0.03).10$$

RESULTS

The values of temperature-humidity regimen and carbon dioxide measured in the incoming and outgoing air during the fattening of broilers under experimental conditions are shown in Tab.1. A characteristic feature was the low daily variation of temperature and relative humidity that did not exceed 3 °C and 8 %, resp., which allowed us to use daily means of these parameters as calculation values throughout the observation period. The range of measured values of CO_2 (0.058—0.142 vol. %) was closely related particularly to the ventilation rate (6.19—1.36 m^3.h^{-1}.kg^{-1}) which, on the other hand, had no essential effect on the temperature and humidity variations in the outgoing air in comparison with those in the incoming air. The temperature-humidity conditions presented in Tab. 1 cannot be related to the field of physiology of thermoregulation processes that are linked to the production of water vapours and carbon dioxide in chickens kept in thermal welfare under infralamps.

Table 2 shows the calculated production of noxious components for 5 weight categories up to the final live weight of 2.0 kg. The mean production of water vapours up to the weight of 0.25 kg.head^{-1} was 6.40 g.h^{-1}.kg^{-1} varying from 7.06 to 6.19 g.h^{-1}.kg^{-1}. With the weight increasing up to 0.5 and 1.0 kg.head^{-1}, this parameter decreased to 6.08 (6.37—5.73) g.h^{-1}.kg^{-1} and 6.14 (6.66—5.66) g.h^{-1}.kg^{-1}, resp. The decrease was yet more marked in broilers in the weight range of 1.0—1.5 kg.head^{-1} (5.46 g.h^{-1}.kg^{-1}, ranging from 5.87 to 5.01) and 1.5—2.0 kg.head^{-1} (4.02 g.h^{-1}.kg^{-1}, ranging from 4.44 to 3.78).

The mean production of carbon dioxide by broilers of the first three weight categories was more or less the same up to the weight of 1.0 kg.head^{-1}, ranging between 1.79 and 1.85 l.h^{-1}.kg^{-1}. The total variance up to this weight

Table 1. The range of calculation values in broilers fattened under experimental conditions

Body weight in kg	Weight categories				
	< 0.25	**0.25—0.5**	**0.5—1.0**	**1.0—1.5**	**1.5—2.0**
Te (°C)					
$\bar{x}$	17.0—17.8	15.4—16.6	15.7—20.2	14.2—16.0	14.8—18.3
R	0.0—2.0	0.0—1.0	1.0—2.5	0.0—1.0	0.0—2.0
RHe (%)					
$\bar{x}$	55.9—63.1	51.2—63.0	50.7—52.9	55.2—62.0	50.2—67.8
R	3.0—7.0	2.0—6.0	3.0—5.0	0.0—6.0	2.0—8.0
Ti (°C)					
$\bar{x}$	19.2—20.6	18.2—19.8	18.1—20.9	16.5—18.8	18.0—20.0
R	1.0—2.5	1.0—2.0	1.0—3.0	1.0—2.5	0.0—3.0
RHi (%)					
$\bar{x}$	57.5—62.2	53.4—64.6	54.6—69.1	63.6—73.0	55.3—69.2
R	2.0—6.0	2.0—8.0	4.0—7.0	0.0—4.0	0.0—8.0
Cci (vol. %)	0.058—0.082	0.082—0.142	0.078—0.113	0.084—0.135	0.082—0.138
Vcu (m^3.h^{-1}.kg^{-1})	3.42—6.19	1.99—4.30	2.14—3.55	1.36—2.87	1.79—2.91

$\bar{x}$ — Daily arithmetic mean; R — Daily variation range

Table 2. Production of water vapours and carbon dioxide by broilers under experimental conditions

Body weight in kg	Weight categories < 0.25	0.25—0.5	0.5—1.0	1.0—1.5	1.5—2.0
Mwu ($g.h^{-1}.kg^{-1}$)					
N	8	7	9	6	6
$\bar{x}$	6.40	6.08	6.14	5.46	4.02
R	7.06—6.19	6.37—5.73	6.66—5.66	5.87—5.01	4.44—3.78
SD	0.42	0.17	0.30	0.26	0.25
V (%)	6.56	2.80	4.90	4.80	6.22
Vcu ($l.h^{-1}.kg^{-1}$)					
N	10	10	10	11	11
$\bar{x}$	1.85	1.81	1.79	1.56	1.23
R	1.94—1.76	1.88—1.70	1.92—1.64	1.91—1.24	1.54—1.02
SD	0.063	0.057	0.074	0.203	0.193
V (%)	3.40	3.15	4.10	13.0	15.7

was 1.94—1.64 $l.h^{-1}.kg^{-1}$. With the weight categories 1.0—1.5 $kg.head^{-1}$ and 1.5—2.0 $kg.head^{-1}$ a decrease to 1.56 (1.91—1.24) $l.h^{-1}.kg^{-1}$ and 1.23 (1.54—1.02) $l.h^{-1}.kg^{-1}$ was observed.

The variations of bioclimate in the hall for intensive fattening of broilers were affected considerably not only by the biological load but also by the external climate. The outside temperatures ranged from –4 to 28.6 °C and relative humidity from 35 to 90.2 % (Tab. 3). Although the temperature in the hall was controlled by means of automated regulation of heating aggregates, its relative humidity ranging from 30.1 to 71.2 % (also within individual weight categories, e.g. from 34.0 to 71.2 % between 0.5 and 1.0 kg. $head^{-1}$) suggested considerable fluctuation of the content of water vapours. Because of that only values measured at relatively stabilised weather and constant ventilation rate could serve as calculation values. The decisive factor affecting the bioclimatological changes including the CO_2 content (0.054—0.303 vol. %) was the intensity of ventilation that varied from 6.69 to 0.62 $m^3.h^{-1}.kg^{-1}$.

The production of noxious components observed in our study (Tab. 4) failed to show decreasing tendency with the increase of body weight. The highest mean production of water vapours (6.48 $g.h^{-1}.kg^{-1}$) was recorded up to the weight of 0.25 $kg.head^{-1}$, ranging from 7.34 up to 5.90 g. $h^{-1}.kg^{-1}$ and then, with irregular small variations, ranging between 5.78 and 6.13 $g.h^{-1}.kg^{-1}$ for the remaining weight categories. The total variation range was 7.18—4.77 $g.h^{-1}.kg^{-1}$. Therefore, when speaking about the real production of water vapours, the value of 6.05 $g.h^{-1}.kg^{-1}$ with broilers of weight up to 2.0 $kg.head^{-1}$ can be considered. Analogically, the mean production of carbon dioxide over the period of observation was on the level of 1.72 $l.h^{-1}.kg^{-1}$. With regard to individual weight categories the production of carbon dioxide showed little fluctuations and ranged from 1.69 to 1.75 $l.h^{-1}.kg^{-1}$. The total variation range was 1.84—1.53 $l.h^{-1}.kg^{-1}$.

DISCUSSION

A high hygiene standard is an important part of active management of health and welfare in intensive animal rearing. Rapidly growing broiler chicks with labile immune status are very sensitive to all negative environmental stimuli and because of that optimization of bioclimate is one of the decisive tasks of breeding and care of animal health.

A starting point of management and regulation of microclimate is the production of noxious components

Table 3. The range of calculation values in broilers under operation conditions

Body weight in kg	Weight categories < 0.25	0.25—0.5	0.5—1.0	1.0—1.5	1.5—2.0
Te (°C)	–4—10.6	5.0—12.5	0.0—28.6	–4.0—28.6	–4.0—26.0
RHe (%)	64.0—90.0	70.4—90.2	35.0—83.2	35.0—90.0	40.0—82.9
Ti (°C)	28.0—29.4	23.8—29.1	21.3—30.0	20.2—28.6	20.0—28.4
RHi (%)	30.1—48.0	44.6—71.1	34.0—71.2	35.1—64.8	43.9—67.2
Cci (vol.%)	0.158—0.226	0.132—0.308	0.058—0.272	0.054—0.075	0.076—0.220
Vu ($m^3.h^{-1}.kg^{-1}$)	0.82—1.40	0.62—1.77	0.72—6.06	0.82—6.69	0.94—3.84

Tab. 4. Production of water vapours and carbon dioxide by broilers under operation conditions

Body weight in kg	Weight categories					
	< 0.25	0.25—0.5	0.5—1.0	1.0—1.5	1.5—2.0	0.05—2.0
Mwu ($g.h^{-1}.kg^{-1}$)						
N	12 + 9*	12 + 6*	21 + 6*	24 + 18*	27 + 9*	96 + 48*
$\bar{x}$	6.48	5.83	6.09	6.13	5.78	6.05
R	7.34—5.90	6.20—4.90	6.75—5.23	7.18—5.22	6.65—4.77	7.34—4.77
SD	0.532	0.448	0.559	0.607	0.560	0.558
V (%)	8.21	7.68	9.18	9.90	9.69	9.22
Vcu ($l.h^{-1}.kg^{-1}$)						
n	12	12	21	24	27	96
$\bar{x}$	1.72	1.75	1.72	1.69	1.73	1.72
R	1.79—1.63	1.80—1.69	1.84—1.53	1.83—1.56	1.83—1.60	1.84—1.53
SD	0.067	0.045	0.096	0.096	0.068	0.074
V (%)	3.89	2.57	5.68	5.68	3.93	4.30

* — registration measurements

which is affected in broilers by complex relationships between dynamic factors of which the most important are metabolism intensity, thermoregulation capability, and locomotory activity of chickens, increasing proportion of excrements in the litter, and thermal-humidity conditions of the internal atmosphere. Therefore the standardized values constitute only the "assumed median" which takes into consideration also other sources present in animal housings. There is a direct relationship between the increase in productivity and the intensity of metabolism and increasing environmental temperature (15).

According to the standard ON 73 4502 (8), still used for air exchange calculations in The Slovak and The Czech Republic, chickens 1—6 weeks old, housed on litter, produce at environmental temperatures of 30 °C and 20 °C up to 9.85 $g.h^{-1}.kg^{-1}$ and 6.03 $g.h^{-1}.kg^{-1}$ water vapours and 1.871 $CO_2.h^{-1}.kg^{-1}$ and 1.21 $CO_2.h^{-1}.kg^{-1}$, resp. The German TGL 29084 (16) is oriented on the live weight of broilers without considering their respective type. The limiting values of production of water vapours at identical thermal conditions are 16.0 $g.h^{1}.kg^{1}$ for chickens weighing 0.05 kg, and 3.55 $g.h^{-1}.kg^{-1}$ for those weighing 2.0 kg. The respective CO_2 values range from 3.61 $l.h^{-1}.kg^{-1}$ to 1.3 $l.h^{-1}.kg^{-1}$. The French standard values (1) range from 12.0 to 4.5 $g.h^{-1}.kg^{-1}$ for water vapours and from 1.86 to 1.301 $l.h^{-1}.kg^{-1}$ for CO_2.

The use of mean values of daily temperatures and relative humidity in the incoming and outgoing air for calculation of production of water vapours in the experiment results in considerable approximation of the calculated and real values as it considers the biorhythm of chickens throughout the fattening cycle. Under the operation conditions with high variations of temperature and humidity with regard to the micro- and macro-climate, the ambulant measurements predominated and the mean values were usable only for the intervals of 6—8 hours. This fact together with some objective errors in determination of volume of the exchanged air and differences in bioclimate within the large hall have undoubtedly a negative effect on the exactness of obtained results.

On the other hand, the large number of animals acted as a compensating factor.

Bieber (2) used the same method of calculation to determine production of water vapours in small-capacity pig houses (40 pigs in preliminary fattening) based on ambulant measurements, and obtained variation coefficient as high as 22 %. The maximum variation coefficients reached in our study were 6.56 % under experimental conditions and 9.90 % under operation conditions.

Our results for production of water vapours and carbon dioxide are related to the weight of birds which is in broiler chickens a more important factor than the age. They also suggest a decisive influence of litter under conventional intensive fattening conditions.

Different situation occurred under experimental conditions. Although the biological load per area unit imitated the operational conditions, changes in locomotory activity of birds involved as a rule the whole group and production of noxious components decreased with the increasing weight (6.40—4.02 $g.h^{-1}.kg^{-1}$; 1.85—1.23 $l.h^{-1}.kg^{-1}$). In the hall with total biological load of 25 000 broilers and irregular activity of birds such a decrease was not observed. With five weight categories up to the final weight of 2.0 kg the production of water vapours ranged between 5.78 and 6.48 $g.h^{-1}.kg^{-1}$ ($\bar{x}$ = 6.05) and that of carbon dioxide between 1.69 and 1.751 $l.h^{-1}.kg^{-1}$ ($\bar{x}$ = 1.72).

The symbols used for Tables (1—4)

Te, RHe	Temperature and relative humidity of the incoming (external) air (°C, %)
Ti, RHi	Temperature and relative humidity of the outgoing (internal) air (°C, %)
Cci	Concentration of carbon dioxide in the outgoing (internal) air (vol.%)
Vu	Ventilation per unit of body weight ($m^3.h^{-1}.kg^{-1}$)
Mwu	Production of water vapours per unit of body weight ($g.h^{-1}.kg^{-1}$)
Vcu	Production of carbon dioxide per unit of body weight ($l.h^{-1}.kg^{-1}$)
x_i, x_e	Specific humidity of the outgoing and incoming air ($g.kg^{-1}$)
$ń_i$	Specific weight of outgoing air ($kg.m^{-3}$)
n	Number of measurements
SD	Standard deviation
V(%)	Variation coefficient (s/x.100)

REFERENCES

1. Anonymous, 1998: La ventilation: objectives, normes et mise en oeuvre. *Sciences & Techniques Avicoles*, Septembre, 17—22.

2. Bieber, H.P., 1975: *Untersuchungen űber die Wasserdampf- und Kohlendioxidbildung in einem einstreulosen Schweinemaststall.* Dissertation Thesis, Freie Universität Berlin, 1975. Journal No. 869, 99 pp.

3. Borchert, K.L., 1961: Űber Grundlagen zur Schaffung eines optimalen Klimas in Hűhnerställen. *Arch. Gefl. K.,* 25, 60—75.

4. Marrufo-Villa, D., Quintana, L.J., Castaneda, S.M., 1999: Effect of positive pressure ventilation on production parameters of broiler fowls in a natural environment house. *Veterinaria — Mexico,* 30, 99—103.

5. Moll, D., 1969: Some facts on controlled environment for broilers. *Poultry Sci.,* 52, 10—15.

6. Morrison, W.D., McMillan, I., Bate, L.A., 1987: Effect of air movement on operant heat demand of chicks. *Poultry Sci.*, 66, 854—857.

7. Novák, P., Novák, I., Košář, K., Doušek, J., Zabloudil, F., 1998: Ventilation as a limiting factor in optimisation of welfare and economy of rearing (In Czech). Proc. *Topical Aspects of Animal Bioclimatology,* Brno, ČR, 43—45.

8. ON 43 45 02, 1978: *Ventilation and heating of animal houses* (In Czech), 50 pp.

9. Osbaldiston, G.W., Sainsbury, D.W., 1964: The effect of ventilation on poultry production. *Br. Vet. J.*, 120, 553.

10. Para, Ľ., Ondrašovič, M., Kočišová, A., Ondrašovičová, O., 1996: Ventilation in chicken fattening (In Slovak). Proc. *Ecology and Vet. Med. III*, UVM Košice, 97—100.

11. Payne, C.G., 1961: Studies on the climate of broilers houses. II. Comparison of ventilating systems. *Br. Vet. J.,* 117, 106—113.

12. Pedersen, S., Thomsen, M.G., 2000: Heat and moisture production of broilers kept on straw bedding. *J. agric. Engng Res.*, 75, 177—187.

13. Poláček, M., 1996: *Biomathematics* (In Slovak). Publ. House DATAHELP, Košice, 256 pp.

14. Simmons, J.D., Lott, B.D., Hannigan, T. E., 1998: Minimum distance between ventilation fans in adjacent walls of tunnel ventilated broiler houses. *Applied Engn. Agric.*, 14, 533—535.

15. Stolpe, J., 1979: *Tierphysiologische Grundlagen der Stallklimagestaltung.* Sonderheft *Tierhygiene Inf.*, 11, 26, 48, 121.

16. TGL 29084, 1981: *Stallklimagestaltung, 12 pp.*

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WHITE BLOOD CELL AND METABOLIC RESPONSES IN DAIRY COWS TO OMENTOPEXY

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SUMMARY

The aim of our study was to investigate the degree of leukocytic and metabolic response to the surgical correction of a left-displaced abomasum (LDA). Twenty dairy cows, aged approximately 4.4 years were used. Blood samples were collected from the jugular vein prior to surgery, immediately after surgery, then 2, 5, 24, and 72 hours after surgery, to determine cortisol, glucose, free fatty acids (FFA), L(+)-lactate, β-hydroxybutyrate, and leukocytes. The surgical intervention (omentopexy) led to a significant elevation in leukocytes, cortisol, glucose, FFA, and lactate in blood. The changes found, which were related to stress response, were seen over a 24 hour-period after the surgery. In contrast, serum concentration of β-hydroxybutyrate slowly decreased over the entire observation period (72 hours). The results show that surgical correction of left abomasal displacement has a short-term stress effect on both leukocytes and energy metabolism of dairy cows.

Key words: dairy cows; left displacement of abomasum; stress; surgery

INTRODUCTION

Under stressful conditions, animals show a variety of adaptive changes or stress responses (2). The responses may include changes in feeding behaviour, hypertension, reproductive dysfunction, inefficient feed conversion, gastric and intestinal ulcers, electrolyte imbalance and immune deficiency (1). All of these changes, especially those of chronic character, can adversely affect health status, reproduction, and production in cattle herds (8). Stress response, characterised by increased production of glucocorticoids and catecholamines, is consequently associated with increased intensity of energy metabolism reflected in elevation of certain substrates in the blood (15).

It was demonstrated that a number of manipulations including transport (11), feed deprivation (6), therapeutic manipulation (14), and surgery (5, 20), increased secretion of cortisol from the adrenal cortex in cattle.

The left displacement of abomasum frequently leads to negative energy balance in cows, that triggers lipomobilisation of the peripheric fat (19). Thus, dairy cows suffering from LDA often develope clinical ketosis and fatty liver syndrome.

Therefore, we decided to design an experiment to find out if the surgical correction of LDA results in additional metabolic stress of dairy cows with LDA.

MATERIALS AND MEHODS

Twenty Holstein-Friesian dairy cows, mean age 4.4 years, admitted for treatment of left abomasal displacement, were used in the study. Abdominal surgery (omentopexy) was performed in a standing position 16—24 hours after admission (4). Isocain (2 % procain-hydrochloride, ASID GmbH, Germany) was used as local anaesthesia. The mean duration of preparation for surgery lasted 30—40 minutes, and the surgery about 60 minutes. Blood samples were drawn from the jugular vein at six time points: prior to surgery, immediately after surgery, then 2, 5, 24, and 72 hours after surgery. The plasma cortisol levels were determined by a radioimmunoassay kit (Coat-A-Count [125I]; Diagnostic Products Cooperation, L.A., USA). Serum concentrations of glucose, free fatty acids, and β-hydroxybutyrate were measured using an automatic analysing system (COBAS MIRA®, Hoffman La Roche, D-79630 Grenzach-Wyhlan). Plasma L(+)-lactate levels were determined

fluorometrically after reaction with L(+)-lactate dehydrogenase. The total and differential leukocyte counts were determined in the whole blood (EDTA).

Statistical analysis was carried out by a one-way analysis of variance. In case the ANOVA model revealed a significant time effect a paired *t*-test was performed to compare pre-surgical values with mean values found after surgery.

RESULTS

The ANOVA revealed significant effect of surgical correction of left abomasal displacement on cortisol, glucose, free fatty acids (FFA), L(+)-lactate, and leukocytes ($p < 0.05$).

After surgery, all dairy cows showed transient increase in plasma cortisol concentration ($p < 0.05$). The maximum plasma cortisol concentrations (38.0 ± 4.84 mg.l^{-1}) occurred 2 hours after surgery (Tab.). Within the following 22 hours, the mean cortisol concentrations declined below the values which were found in the cows before surgery.

The surgical stress had stimulating effects on the blood concentrations of glucose, free fatty acids, and L(+)-lactate. The maximal concentrations of them were seen immediately after surgery ($p < 0.05$), after which they decreased, and at 72 hours after surgery they were below the values which were seen before surgery (Tab.). In contrast to glucose, FFA and lactate, β-hydroxybutyrate concentrations slightly declined after surgery and the minimum serum β-HB occurred 24 hours after surgery.

The numbers of white blood cells and their differentiation were affected by surgical stress ($p < 0.05$). A marked leukocytosis as a result of neutrophilia, seen after surgery, returned to presurgical levels within 24 hours (Tab.). The surgery caused the actual percentage of lymphocytes in circulation to decrease, but their absolute number was not significantly affected.

DISCUSSION

The average plasma cortisol levels of 1—10 mg.l^{-1} found in dairy cows during the first month after calving (12) correspond with the values we measured before, then 24 and 72 hours after surgery. The significant effect of surgery on the increase in plasma cortisol levels was similar to that previously reported in cattle after laparotomy (10).

The pattern of cortisol concentrations seen after surgery in this study in dairy cows was similar to the pattern seen in 5—6 month-old cattle after amputation dehorning (20).

Adrenal corticosteroids are known to have immunosuppressive actions (7). In domestic animals, both acute stress conditions, like transportation, or administration of ACTH produce characteristic changes in the population of blood leukocytes and affect lymphocyte functions. In our study, surgical stress resulted in changes in total and differential white blood cell count that are consistent with the changes reported previously in cattle (17). There was a leukocytosis due to a neutrophilia and no significant change in the absolute number of lymphocytes. Increased number of neutrophils in the circulation after stress or after the administration of ACTH or glucocorticoids has been shown to be due to the input of mature neutrophils from the bone marrow storage pool, and to reduced margination of neutrophils (3).

The 24-hour-increase of glucose concentrations seen after surgery can be explained as a reaction of organisms to catecholamine and cortisol rise in the circulation. The exiting glucose is an exclusive energy source for the red blood cells and portions of central nervous system during periods of stress (15). Similarly to our results, a short-term rise of glucose, lasting approx. 6 hours, was seen in cattle after transportation (9). However, the glucose levels are not useful as reliable indicators

Table. Concentrations of cortisol, glucose, free fatty acids (FFA), β-hydroxybutyrate (β-HB), L(+)-lactate, and total (WBC) and differential leukocyte counts in operated dairy cows (mean ± SE)

Index		Sampling time: before surgery	immediately AS	2 hours AS	5 hours AS	24 hours AS	72 hours AS
cortisol	mg.l^{-1}	8.41 ± 1.62	36.4 ± 5.83*	38.0 ±4.84*	19.7 ±2.99*	4.62 ± 0.72	2.91 ± 0.59*
glucose	mmol.l^{-1}	3.31 ± 0.26	5.01 ± 0.43*	4.58± 0.37*	4.08± 0.34*	3.71 ± 0.2	2.84 ± 0.25
FFA	mmol.l^{-1}	1.58 ± 0.18	2.51 ± 0.19*	1.61 ± 0.16	1.67 ± 0.18	1.27 ± 0.09	0.81 ± 0.11*
β-HB	mmol.l^{-1}	3.48 ± 0.52	3.24 ± 0.47	2.88 ± 0.48	2.96 ± 0.5	1.95 ± 0.37	1.98 ± 0.36
lactate	mmol.l^{-1}	0.87 ± 0.17	1.41 ± 0.12*	1.33 ± 0.16	0.91 ± 0.06	0.51 ± 0.04	0.53 ± 0.06
WBC	G.l^{-1}	7.21 ± 0.46	6.53 ± 0.47	9.32 ± 0.69	11.0 ±1.02*	7.04 ± 0.35	6.81 ± 1.99
neutrophils	%	46.0 ± 3.16	48.2 ± 2.66	63.6± 2.00*	66.6± 3.20*	50.4 ± 2.18	38.8 ± 2.25
lymphocytes	%	50.0 ± 3.07	49.3 ± 2.22	35.3 ±1.95*	32.2 ±2.90*	47.5 ± 2.42	58.0 ± 2.31

AS — after surgery; * — mean differs from pre-surgery mean ($p < 0.05$)

of stress intensity in animals as there are many factors which can variously affect blood glucose concentrations (18). The surgery resulted in transient elevation of free fatty acids which were higher than physiological values already seen after surgery (16). The lipomobilisation seen in this study was probably due to effects of catecholamines. Catecholamine rise might have been responsible for the increase of plasma lactate in operated cows as noradrenaline precipitates tissue hypoxia with consequent changes in tissue acid base balance (13). The high plasma concentrations of β-hydroxybutyrate during the whole experiment reflected energy deficiency in dairy cows which was basicaly due to a decreased feed intake during the illness. In agreement with the fact that glucocorticoids exhibit strong antiketogenic effects (6) a slight decline of β-hydroxybutyrate levels was recognised in this trial during the period after surgery.

In conclusion, these data extend existing knowledge by indicating that surgical correction (laparotomic omentopexy) of left displacement of abomasum triggers typical stress reaction, which produces significant changes in blood metabolite and hormone concentrations. Interestingly, glucogenic response was also seen in animals suffering from energy deficiency. When the laboratory data are assessed to make a diagnosis, possible effects of stress response on energy metabolites and blood cells should be considered.

REFERENCES

1. Alams, M. G. S., Dobson, H., 1986: Effect of various veterinary procedures on plasma concentrations of cortisol, luteinizing hormone and prostaglandine $F_2\alpha$ metabolite in the cow. *Vet. Rec.*, 118, 7—10.

2. Breazile, J. E., 1988: The physiology of stress and its relationship to mechanisms of disease and therapeutics. *Veter. Clin. N. Amer. Fd. Anim. Pract.*, 4, 441—480.

3. Carlson, G. P., Kaneko, J. J., 1976: Influence of prednisolone on intravascular granulocyte kinetics of calves under nonsteady state conditions. *Am. J. Vet. Res.*, 37, 149—151.

4. Dirksen, G., 1967: Gegenwärtiger Stand der Diagnostik, Therapie und Prophylaxe der Dislocatio abomasi sinistra des Rindes. *Dtsch. Tierärztl. Wschr.,* 74, 625—633.

5. Fisher, A. D., Knight, T. W., Cosgrove, G. P., Death, A. F., Anderson, C. B., Duganzich, D. M., Matthews, L. R., 2001: Effects of surgical or banding castration on stress responses and behaviour of bulls. *Aust. Vet. J.*, 79, 279—84.

6. Furll, M., Kirbach, H., Knobloch, B., 1993: Glukokortikosteroideinfluss auf die fastenstimulierte Lipolyse und die Leberfunktion bei Kuhen. *Tierärztl. Prax.*, 21, 399—403.

7. Griffin, J. F. T., 1989: Stress and immunity: a unifying concept. *Vet. Immunol. Immunopathol.*, 20, 263—312.

8. Hadbavný, M., Korim, P., Bugarský, A., Hadbavný, M. Jr., Korimová, J., Červená, K., 2002: Effects of nutrition on economical aspects of dairy production (In Slovak). *„Dni výživy a veterinárnej dietetiky“*, 11.–12. 9. 2002, Hrádok pri Jelšave, SR, 98—102.

9. Hartmann, H., Meyer, H., Steinbach, G., Finger, B., 1973: Zu Reaktion des Kälberorganismus auf Transportbelastungen. *Mh. Vet.-Med.*, 28, 647—651.

10. Janowski, T., Grunert, E., Zaremba, W., 1983: Untersuchungen über den Steroidhormonspiegel bei hochtragenden, im Abstand von 1 bis 2 Tagen zweimal laparomierten Kuhen der Rasse „Deutsche Schwarzbunte”. *Zuchthygiene (BERL.)*, 18, 193—202.

11. Locatelli, A., Sartorelli, P., Agnes, F., Bondiolotti, G. P., Picotti, G. B., 1989: Adrenal response in the calf to repeated simulated transport. *Br. Vet. J.*, 145, 517—522.

12. Madej, A., Oyedipe, E. O., Edqvist, L.E., Kindahl, H., 1992: Hormonal interrelationships in postpartum suckled dairy cows. *Acta Vet. Scand.*, 33, 261—271.

13. Nagy, O., Sedovic, M., Slanina, L., 1994: Central and peripheral arterial and venous blood in cattle with respect to acid-base balance evaluations (In Slovak). *Vet. Med.-Czech*, 39, 1—9.

14. Nakao, T., Sato, T., Moriyoshi, M., Kawata, K., 1994: Plasma cortisol response in dairy cows to vaginoscopy, genital palpation *per rectum* and artificial insemination. *J. Vet. Med. A*, 41, 16—21.

15. Nockels, C. F., 1990: Mineral alterations associated with stress, trauma, and infection and the effect on immunity. *Cont. Educ. Pract. Vet.*, 12, 1133—1139.

16. Reichel, P., Kovac, G., Paulikova, I., 1992: Liver fat content and selected biochemical indices of blood in dairy cows. *Biopharm.*, 2, 169—175.

17. Roth, J. A., Kaeberle, M. L., Hsu, H. H., 1982: Effects of ACTH administration on bovine polymorphonuclear leukocyte function and lymphocyte blastogenesis. *Am. J. Vet. Res.*, 43, 412—416.

18. Scholz, H., 1990: Stoffwechselkontrolle in der Milchkuhherde an Hand von Blut- und Milchparametern. *Prakt. Tierärzt., Coll. Vet.*, 21, 32—35.

19. Stober, M., Scholz, H., 1991: Therapie des Lipomobilisationssyndroms der Milchkuh. *Mh. Vet.-Med.*, 46, 563—566.

20. Sylvester, S. P., Stafford, K. J., Mellor, D. J., Bruce, R. A., Ward, R. N., 1998: Acute cortisol responses of calves to four methods of dehorning by amputation. *Aust. Vet. J.*, 76, 123—126.

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THE MICROBIOLOGY AND SHELF-LIFE OF CARP FILLETS STORED IN DIFFERENT CONDITIONS

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ABSTRACT

The microbiological and sensory changes of carp fillets in different storage conditions were studied. A total number of 80 packaged and unpackaged fish samples from freshly killed carp were used. Samples were stored at 3 °C and 15 °C. Mesophilic viable counts, psychrotrophic viable counts, coliform bacteria, enterococci and *Escherichia coli* were examined during storage. A numeric scale from 1—3 was used for the sensoric analysis. The samples were evaluated for appearance, texture, colour and odour. Mesophilic counts and psychrothropic counts reached 10^9 cfu.g^{-1} and 10^6 cfu.g^{-1}, when stored unpackaged at 15 °C. Lower counts were found when the samples were packaged. The fillets were unacceptable for consumers after 3 to 6 days of storage. Storage of the fillets at 3 °C was more acceptable than that above. Mesophilic viable counts and psychrotrophic viable counts reached 1.8.10^9 and 5.6.10^8 cfu.g^{-1} (unpackaged samples) and 1.2.10^7 and 1.3.10^6 cfu.g^{-1} (packaged samples) after 14 days storage. The judges considered samples unfit for consumers after 12 days of storage. Counts of coliform bacteria, *Escherichia coli* and enterococci reached 10^9, < 10^6 and 10^6cfu.g^{-1} for unpackaged samples during storage at 15 °C. Packaging decreased the counts of these bacteria. At 3 °C there were counts 10^7 cfu.g^{-1} for coliforms, 10^4—10^5cfu. g^{-1}for *E. coli* and 10^5 cfu.g^{-1} for enterococci.

Key words: fish; microbiology; sensory panel; storage conditions

INTRODUCTION

The microbiology of fresh fish is reflected by the waters from which it is taken. The sanitary quality of the waters is the key to the overall microbiological quality of the finished products. It is generally accepted that the flesh of newly caught healthy fish is sterile but bacteria are found in variable numbers in three sites on a fish: the slime coat, the gills and the intestines. The numbers of bacteria differ according to the place of their occurrence. The highest levels are found in the intestine. The bacteria can multiply during storage and cause the fish to spoil. The change in fish quality as determined by sensory properties follows increases in microbiological counts, indicating that bacterial load can serve as a useful and objective indicator of gross spoilage (1).

Gonzales *et al.*(5) have studied the microbiology of wild and farmed fresh water fish during iced storage. They have identified 106 strains of Gram-negative, nonmotile, aerobic bacteria. *Psychrobacter, Acinetobacter, Moraxella* and *Chryseobacterium* were dominant genera. *Psychrobacter* sp. and *A. johnsonii* were found in river water as well.

Aeromonas spp. are common contaminants of fish and seafood. They are also ubiquitous in a water environment. *Aeromonas* spp. were identified in 27 (93 %) of fish, in 17 (100 %) fish-egg, in 2 (16 %) of shrimp samples and in 23 (100 %) fresh water samples (7).

Normal microflora in the intestinal tract of fish inhabiting fresh-water reservoirs include lactic bacteria. The number of bacteria depends on the animal species, the composition of food, the age and the season. The highest number of these microorganisms is found in carp (9). Enteric bacteria, such as *Proteus, Citrobacter* and *Providentia* were found to the prevalent particularly in the gills and intestines of macretable fish of the Volga and Caspian Sea (10).

The activity of undesirable contaminating microbiota might be attributed to the occurrence of high amounts of biogenic amines. Bacterial growth and histamine formation in Pacific mackerel during storage at 0, 4, 15 and 25 °C have been monitored by Kim *et al.* (8). Histamine was found in the muscle

when fish were stored above 4 °C, and aerobic plate counts reached 10^6 cfu.g^{-1}. When fish were unsuitable for the consumers, toxicological levels of histamine were always found.

The objectives of this work were to determine the bacterial contamination and the sensory quality of the carp during different storage conditions.

MATERIAL AND METHODS

Samples

The fish samples were obtained from freshly killed carp (*Caprinus carpio*) of an average body mass 2.3 kg. The fish was drawn and after beheading and trimming away the fish tail, the body was cut into fillets. A total number of 80 samples was investigated. Half of the fillets were packaged in plastic bags and the air was partially drained, the other half were laid unpackaged on a plate. The fillets were prepared and packaged in a fish-processing plant and immediately transported to the laboratory. All the samples were stored at 3 °C and 15 °C. A temperature of 3 °C is recommended for fish meat storage as a relatively safe temperature regime. In order to simulate situations when the temperature control fails, we stored samples at 15 °C. The time of storage was 6 days for 15 °C and 14 days for 3 °C. The time of storage was determined by the rate of fish spoilage. The samples were analysed in duplicates after every 24 hours.

Sensory analysis

The sensory evaluation panel consisted of five trained panellists. The samples were evaluated for appearance, texture, odour and colour. A numeric scale from 1–3 was used.

1 – fresh appearence, elastic muscle, light pink colour and typical fish odour;

2 – thin layer of slime on the surface of the fillets, pink grey colour of the muscle less elasticity, typical fish odour with a slightly strange odour;

3 – thick layer of the slime on the surface of fillets, the muscle soft and grey, off-odour.

Microbiological analysis

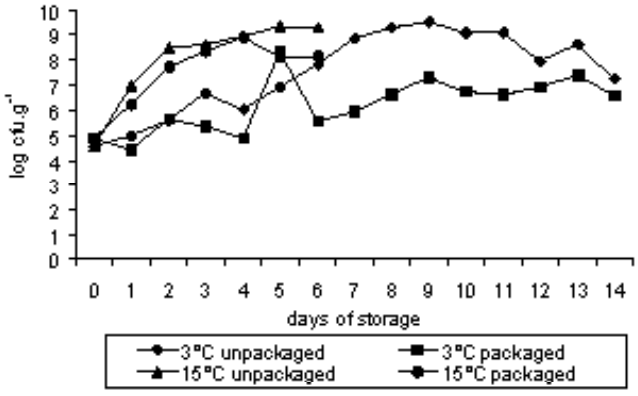
The mesophilic viable count (MVC), psychrotrophic viable count (PVC), coliform bacteria count, and count of *Escherichia coli* and enterococci were investigated. Ten grams of fish muscle were homogenised with 90 ml of diluent. 1:10 serial dilutions were used for the microbiological analysis. MVC were performed in poor plates with Plate-Count Agar (HiMedia India) according to CSN ISO 4833 (13). The plate count method and Plate Count agar (HiMedia, India) were used for the determination of PVC. The plates were incubated for 7 days at 4 °C. Coliform bacteria count was confirmed according to the CSN ISO 4832 (14). The violet Red Bill agar was used (HiMedia, India).

The counts of *Escherichia coli* were determined using TBX medium (Oxoid Ltd.) and counts enterococci were performed on Slanetz-Bartley agar (HiMedia, India). The plates were incubated at 37 °C for 24—48 hours. The STAT Plus programme was used for the statistical evaluation.

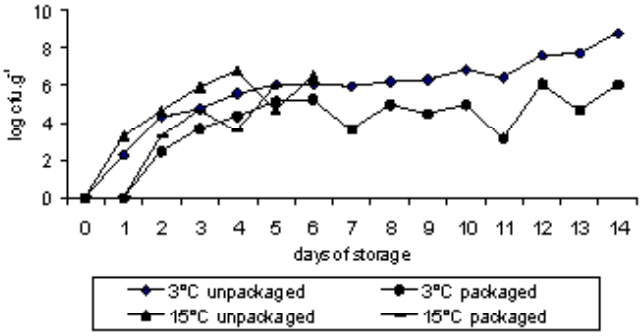
RESULTS

Eighty samples of fish fillets were investigated. Mesophilic viable counts of unpackaged fillets increased during storage at 3 °C 14 days from the initial concentration $3.1.10^4$ cfu.g^{-1} to $1.8.10^9$ cfu.g^{-1}. The packaged fillets (in the same storage conditions) showed $3.9.10^6$ cfu.g^{-1} at the end of storage (14 days). The fish which were stored at 15 °C reached MVC 2.10^9 cfu.g^{-1} during the 6 days. Lower numbers were found in packaged fillets ($1.4.10^8$ cfu.g^{-1}) (Graph 1).

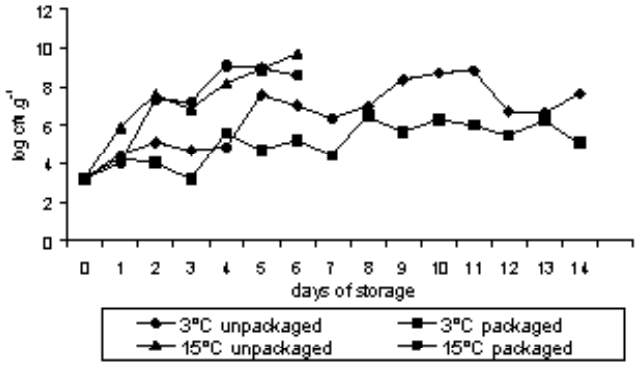
The counts of the psychrotrophic bacteria were lower than MVC and at 3 °C reached cfu. $5.6.10^8$ g^{-1} (unpackaged samples) and $1.3.10^6$ cfu.g^{-1} (packaged samples) and when stored at 15 °C: 3.10^6 and $1.2.10^6$ cfu.g^{-1} (Graph 2).



Graph 1. The development of mesophilic bacteria in carp fillets



Graph 2. The development of psychrotrophic bacteria in carp fillets

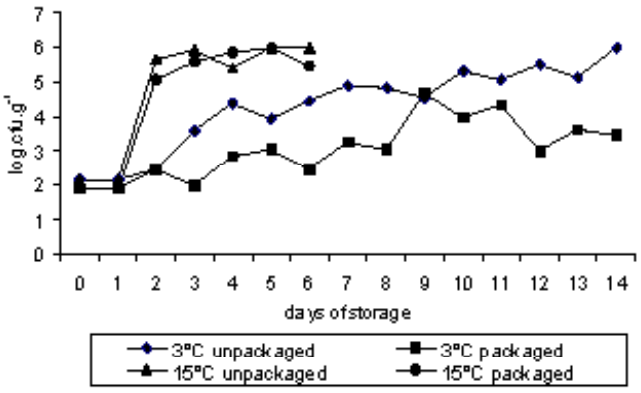


Graph 3. Coliform bacteria in carp fillets (development)

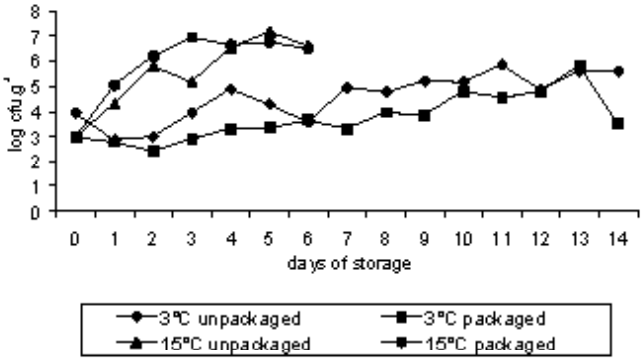
Tab. 1. The statistical evaluation of microbial findings in carp fillets

Microorganisms	Sample/temperature	n	Mean log cfu.g^{-1}	s log cfu.g^{-1}	Student *t*-test
MVC	unpackaged/3 °C	15	7.485	1.638	p = 0.01
	packaged/3 °C	15	6.005	0.943	
	unpackaged/15 °C	7	8.028	1.726	statistically
insignificant					
	packaged/15 °C	7	7.575	1.498	insignificant
PVC	unpackaged/3 °C	15	5.657	2.183	p = 0.05
	packaged/3 °C	15	4.080	1.604	
	unpackaged 15 °C	7	4.534	2.342	statistically
insignificant					
	packaged/15 °C	7	3.378	2.548	insignificant
Coliform bacteria	unpackaged/3 °C	15	6.341	1.989	p = 0.05
	packaged/3 °C	15	5.044	1.139	
	unpackaged/15 °C	6	7.247	2.300	statistically
insignificant					
	packaged/15 °C	8	6.755	2.216	insignificant
Escherichia coli	unpackaged/3 °C	14	4.434	1.093	p = 0.01
	packaged/3 °C	14	3.159	1.182	
	unpackaged/15 °C	6	5.183	1.508	statistically
insignificant					
	packaged/15 °C	6	4.910	1.519	insignificant
Enterococci	unpackaged/3	14	3.733	0.923	p = 0.01
	packaged/3	14	6.343	1.519	
	unpackaged/15	7	3.787	0.711	p = 0.01
	packaged/15	7	5.651	1.229	

MVC — mesophilic viable counts; PVC — paxheoreophic viable counts



Graph 4. *Escherichia coli* in carp fillets (development)

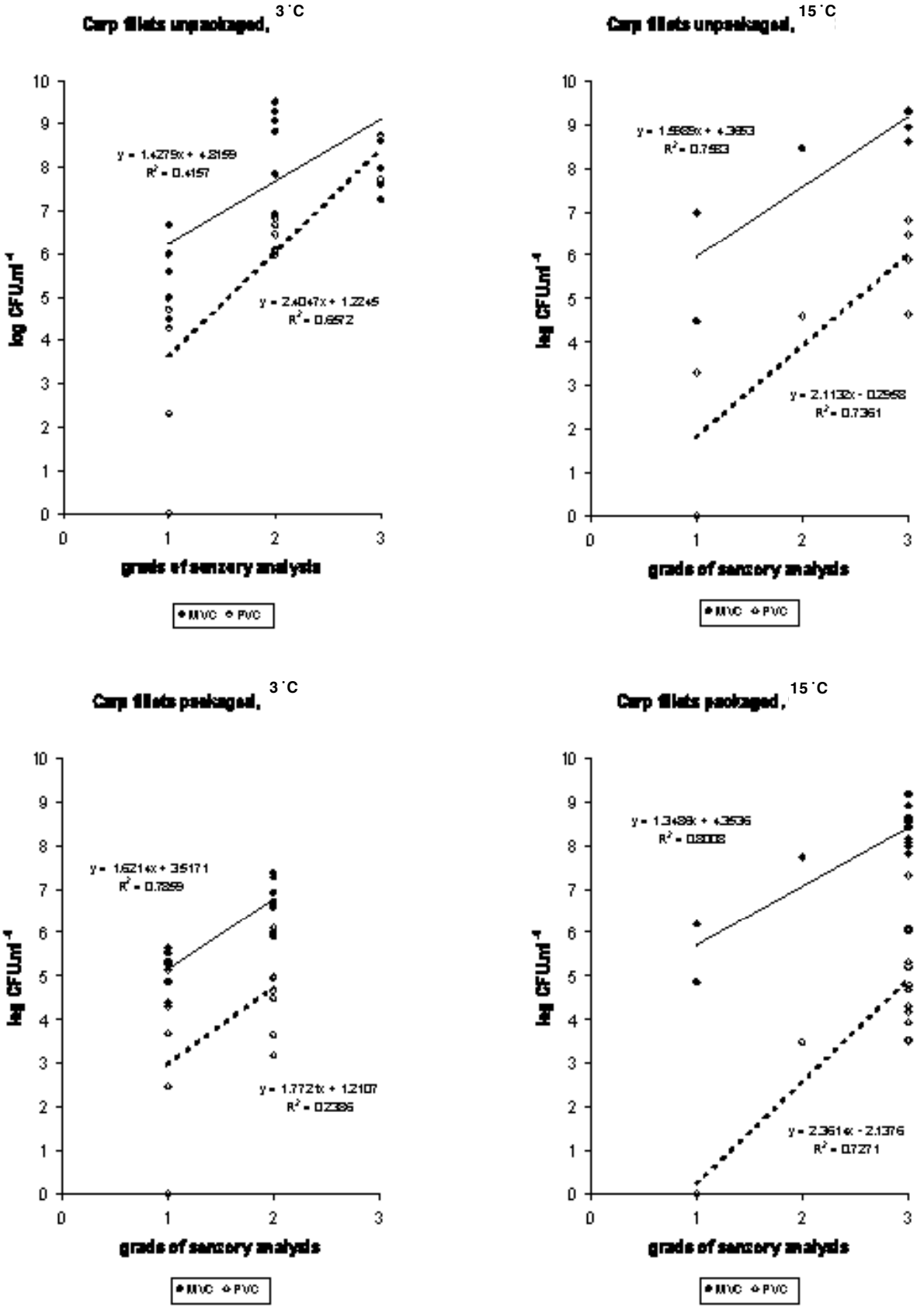


Graph 5. Enterococci in carp fillets (development)

The effect of the storage of carp fillets on coliformes is demonstrated in Graph 3. The initial counts of coliformes were $1.5.10^3$ cfu.g^{-1} and these counts rose continuously, reaching about $4.5.10^9$ cfu.g^{-1} (15 °C unpackaged samples) and 9.10^8 cfu.g^{-1} (packaged samples) and 4.10^7 cfu.g^{-1} and $1.2.10^5$ cfu.g^{-1} (3 °C).

The increase of *Escherichia coli* was observed as well but the counts were considerably lower as compared with coliformes (Graph 4). The initial count of enterococci was 10^3 cfu.g^{-1}.10^6.cfu g^{-1} was reached after the storage of unpackaged and packaged samples 6 days at 15 °C (Graph 5). *Enterococci* rose at 3 °C to 4.10^5 and 8.10^3 cfu.g^{-1} (Graph 5). The statistical evaluation is shown in Tab. 1.

The organoleptic properties of the stored fish were evaluated in a relationship with all tested groups of the organisms. Regression analysis was used for statistical evaluation. A statistically important relationship was found between the sensoric evaluation and MVC and *E. coli* (15 °C, unpackaged) (Graph 6).



Graph 6. Statistical evaluation of the relationship between MVC, PVC and sensory analysis (regression analyse)

DISCUSSION

Most of the microbial studies dealing with fish have concentrated on marine varieties and there is little information about fresh water fish. The microbiological quality of the fish and finished products depends on the water sources from which these animals were taken and on the various processing steps such as peeling, shucking, evisceration breading and the like. In a study of haddock fillets, most microbial contamination was found to occur during filleting and subsequent handling prior to packaging (12).

The most important factor affecting the shelf life of fish is storage temperature. During the storage of carp fillets for 6 days at 15 °C both mesophilic bacteria reached 10^9 and 10^8 cfu.g^{-1}and psychrotropic bacteria 10^6 cfu.g^{-1}. The fillets were rated not acceptable for consumption (grade 3). These bacteria were significantly lower ($p<0.05$) when the fillets were stored at 3 °C.

After 6 days of storage the sensory properties were not excellent but still acceptable for consumers (grade 1—2). Grade 3 by sensory panel was observed already after 12 days of storage. Keeping the fish at 3 °C can prolong the storage before spoilage by some days compared with those kept at 15 °C. Similar results were obtained also by Gelman *et al*. (4). They evaluated the sensory and microbiological characteristics of pond-raised freshwater silver perch fish during cold storage.

Packaging preserves food from secondary contamination. The packaging of fillets in polyethylene bags with a partial draining of the air reduced the numbers of mesophilic and psychrotrophic bacteria about 1 log to 3 log in g^{-1} of fish. Lyhs *et al.* (11) have studied the microbiolocal quality and shelf life of vacuum packaged gravad rainbow trout stored at 3 and 8 °C. They have presented results similar to ours.

Microbiological analysis confirmed the presence of indicative bacteria. The counts of coliformes, enterococci, and *Escherichia coli* were lower than the other bacteria counts determined at the time of storage. Significant differences in coliformes ($p<0.01$), enterococci ($p<0.01$) and *E. coli* ($p<0.01$), due to temperature effects during storage of the fillets in a bag, were observed. No differences in coliformes were found in unpackaged fillets. An increase of Enterobacteriaceae in salmon slices and fresh trout fillets during the shelf life was also observed by Gonzales *et al. (6).*

The fluctuation of the number of microorganisms during the storage can be due to different initial contamination of the fillets. The extent of the growth of microorganisms, such as Enterobacteriaceae, enterococci, and others may be responsible for the formation of the biogenic amines in fish meat and for hygienic problems in fishfood (3, 2). The safety of fresh and frozen fish is ensured by directive No. 91/1999 (15), where the determined limit for the total viable counts of microorganisms is 10^5 cfu. g^{-1} and for Escherichia coli 10^2cfu.g^{-1}.

CONCLUSION

The microbiology of carp fillets during different storage conditions was studied. High bacterial counts had a major effect on the sensory quality of fish meat. The change in fish quality as determined by sensoric score was followed by an increase of bacteria counts in fillets. Critical counts of bacteria 10^8cfu.g^{-1} (for mesophiles) and 10^6cfu.g^{-1} (for psychrotrophs) corresponded with sensory evaluation. For consumers, the fillets were not acceptable on the second day of storage at 15 °C. During storage at 3 °C they were acceptable for six days. According to directive no. 326/2001 of the Czech Ministry of Agriculture (16) it is recommended to store fish at 2 °C.

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REFERENCES

1. Du, W. X., Kim, J., Cornell, J. A., Huang, T., Marshall, M. R., Wei, C. I., 2001: Microbial, senzory, and electronic nose evaluation of yellowfin tuna under various storage conditions. *J. Food Prot*., 64, 2027—2036.

2. Durlu-Özkaya, F., Kamuran, A., Nilüfer, V., 2001: Biogenic amines produced by *Enterobacteriaceae* isolated from meat products. *Meat Science,* 58, 163—166.

3. Gardin, F., Martuscelli, M., Caruso, M. C., Galgano, F., Crudele, M. A., Favati, F., Guerzoni, M. E., Suzzi, G., 2001: Effect of pH, temperature and NaCl concentration on the growth kinetics, proteolytic activity and biogenic amine production of *Enterococcus faecalis*. *Int. J. Food Microbiol*., 64, 105—117.

4. Gelman, A., Glatman, L., Drabkin, V., Harpaz, S., 2001: Effects of storage temperature and preservative treatment on shelf life of pond-raised freshwater fish, silver perch (*Bidyanus bidyanus*). *J. Food Prot*., 64, 1584—1591.

5. Gonzales, C. J., Santos, J. A., Garcia-Lopez, M. L., Otero, A., 2000: Psychrobacters and related bacteria in freshwater fish. *J. Food Prot*., 63, 315—321.

6. Gonzales-Rodriguez, M. N., Sanz, J. J., Santos, J. A., Otero, A., Garcia-Lopez, M. L., 2001: Bacteriological quality of aquacultured freshwater fish portions in prepackaged trays stored at 3 degrees C. *J. Food Prot*., 64, 1399—1404.

7. Hanninen, M. L., Oivanen, P., Hirvela-Koski, V., 1997: *Aeromonas* species in fish, fish-eggs, shrimp and freshwater. *Int. J. Food Microbiol*., 34, 17—26.

8. Kim, S. H., Field, K. G., Chang, D. S., Wei, C. I., An, H., 2001: Identification of bacteria crucial to histamine accumulation in pacific mackerel during storage. *J. Food Prot*., 64, 1556—1564.

9. Kvasnikov, E. I., Kovalenko, N. K., Materinskaya, L. G., 1977: Lactobacilli of freshwater fishes (In Russian) *Mikrobiologiya*, 46, 755—760.

10. Lartseva, L. V., Rogatkina, I. I., Bormotova, S. V., 1996: Enterobacteria isolated from commercial fishes from the Volga-Caspian basin (In Russian). *Gig. Sanit*., No. 3, 22—23.

11. Lyhs, V., Lahtinen, J., Fredriksson-Ahomaa, M., Hyytiä-Trees, E., Elfing, K., Korkeala, H., 2001: Microbiological quality and shelf-life of vacuum-packaged "gravad" rainbow trout stored at 3 and 8 °C. *Int. J. Food Microbiol.*, 70, 221—230.

12. Nickerson, J. T. R., Goldblith, S. A., 1964: A study of microbiological quality of haddock fillets and shucked, soft-shelled clams processed and marketed in the greater Boston area. *J. Milk Food Technol.*, 27, 7—12.

13. CSN ISO 4832, 1995: Microbiology — General guidance for the enumeration of coliformes — Colony count technique (In Czech).

14. CSN ISO 4833, 1995: Microbiology — General guidance for the enumeration of micro-organisms — Colony count technique at 30 °C (In Czech).

15. Vyhláška 91/1999 Sb. Ministerstva zdravotnictví, kterou se mění vyhláška Ministerstva zdravotnictví č. 294/1997 Sb. o mikrobiologických požadavcích na potraviny, způsobu jejich kontroly a hodnocení. *Sbírka zákonů,* 1999 č. 91: 2440—2455 (In Czech).

16. Vyhláška MZ 326/2001, kterou se provádí § 18 písm. a), d), g), h),i) a j) zákona č. 110/1997 Sb. o potravinách a tabákových výrobcích a o změně a doplnění některých souvisejících zákonů, ve znění pozdějších předpisů, pro maso, masné výrobky, ryby, ostatní vodní živočichy a výrobky z nich, vejce a výrobky z nich. *Sbírka zákonů,* 2001 č. 126, 7414—7442 (In Czech).

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A STUDY OF THE NEURAL MECHANISMS UNDERLYING HYPERALGESIA IN RAT

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ABSTRACT

Hyperalgesia is an altered state of cutaneous sensation characterized by an increase in the pain evoked by a noxious stimulus. We studied the effects of a thermally evoked hind paw withdrawal latency and paw elevation time in normal rats and in rats with a hind paw rendered unilaterally hyperesthetic by the unilateral application of loose ligatures of the sciatic nerve. The data suggests that the neural mechanisms underlying hyperalgesia are based on activation of different modality — specific excitatory amino acid receptor types and subsequently, a unique second mesenger system in the spinal cord.

Key words: hyperalgesia; light touch; paw; peripheral nerve injury; rat; sciatic nerve

INTRODUCTION

Somatic and visceral sensory signals relay information from an organism's external and internal environment and make essential contributions to homeostasis and behavior (8). In all sensory systems, prolonged or repeated application of an adequate stimulus leads to reduction in the activity of the receptor adaptation. This is also true of nociceptors (5). Polymodal C-nociceptors adapt within a few seconds to prolonged heat stimuli and they show fatigue with repeated stimuli even at intervals of several minutes. In contrast to other sensory systems, however, the pain system is also capable of enhancing its responsiveness as a consequence of adequate stimulation.

This process is called sensitization and is characterised by a lowered response threshold, induction of spontaneous activity and enhanced responses in the suprathreshold range (3). This gives the condition we call *hyperalgesia*, which is characterised by a lowered pain threshold, increased pain from suprathreshold stimuli and occasionally spontaneous pain (7). *Hyperalgesia* is consequent on a number of types of tissue injury and is a prominent feature of diseases of the nervous system and inflammation generally.

There are two types according to the location relative to the injury site. *Primary hyperalgesia* refers to the site of injury and is characterized by enhanced responsiveness to mechanical and heat stimuli, and *secondary hyperalgesia* refers to changes in the surrounding undamaged skin and is characterized by enhanced responsiveness to mechanical stimuli only (8).

To gain a better understanding of this phenomenon, this study evaluated the effects on the thermally evoked hind paw withdrawal latency in normal rats and in rats with a hind paw rendered unilaterally hyperesthetic by the unilateral application of loose ligatures of the sciatic nerve.

MATERIAL AND METHODS

All procedures were reviewed by the local animal care committee and were consistent with the IASP guidelines. Twelve adult male rats weighing between 280—300 g were used who had been allowed at least five days to acclimatize to the laboratory prior to use. The rats were allowed free access to food and water during the experiment except during behavioral testing. The room temperature was maintained at 20—22 °C.

Sciatic nerve ligation

The hyperesthetic state was induced by an incomplete ligation of the sciatic nerve. The animals were anaesthetized with an intraperitoneal injection of sodium pentobarbital 50 $mg.kg^{-1}$. The level of anaesthesia was checked periodically by examining pupil size and reflexes. Rectal temperature was kept between 37—38 °C by a servo-controlled heating blanket.

After a skin incision, the *biceps femoris* was bluntly dissected at midthigh to expose the sciatic nerves. In those animals which would develop the partial constriction neuropathy, four sutures were wrapped around the nerve and tied with a square knot about five millimetres proximal to sciatic trifurcation. The sutures were drawn tight enough to closely envelope the nerve yet remained moveable along the nerve so as not to occlude the perineural blood flow.

Following placement of the sutures, the muscle was closed in layers using 4—6 silk sutures and the skin was closed with several nine millimetre stainless steel wound clips. A sham surgery identical to that performed on the injured limb was performed on the contralateral limb for each animal, however, the sciatic nerve was not constricted. The animals were then allowed to recover under a warm lamp and were monitored routinely to ensure general health.

Behavioral testing

The paw withdrawal latency (PWL) and paw elevation time (SPET) was determined. Animal was allowed 15—20 minutes for acclimatization to the clear 10 × 20 cm × 2 mm whole thick glass cage prior to any testing. A radiant heat source (halogen projector lamp 50 W, 8 V) was contained in a movable holder placed beneath the glass floor. The radiant heat sources aperture was four millimetres in diameter. The voltage to the thermal source was controlled by a constant voltage supply. To reduce variability in the plate surface caused by room temperature, the interior of the box under the animal was prepared with a heat source such that the under plate temperature was regulated to 30 °C.

The calibration of the thermal test system was such that the average response latency in normal untreated rats, measured prior to the initiation of an experimental series, was ten seconds. The under floor heat source was positioned in such a manner that it focused at the plantar surface of one hind paw where it was in contact with the glass. Care was taken not to focus the light source on the skin that was off of the glass plate. The light was then activated. The activation of the light initiated a timing circuit. The on off switch to heat source was also controlled by a digital timer.

The data for each paw at each particular testing period were recorded from the average of five separate trials, taken at 15—20 minute intervals. PWL and SPET was determined for each animal prior to induction of neuropathic injury (day 0), day 3 after surgery, and then weekly thereafter up to four weeks following injury. All behavioral testing was conducted between nine and twelve hours for each testing day. For statistical analyses, changes in PWL and SPET measurements were expressed as difference scores. Thus, the time of paw withdrawal or paw elevation on the normal side is compared with a similar measurement on the operated side, and the difference recorded. A negative difference score in PWL thus implies that the affected paw is withdrawn more quickly and a positive difference score in SPET implied that the affected paw is elevated longer after each mulation.

RESULTS

Our researches were similar to that described in other published reports (1, 6). Mean diference scores (operated — sham operated sites) were established for each animal on day 0 (baseline) and on days 3, 7, 14, 21, and 28 postsurgery. On day 0, the difference scores were not significantly different. The thresholds were similar for both hindpaws. The differences in PWL and SPET initiate that both measured parameters quickly rise to a maximum and then resolve. Thus PWL and sustained SPET are maximal at approximately three days following placement of the sutures and then steadily improve. PWL returning to normal between twenty-eight days and SPET between twenty-one days following the lesion.

The negative difference scores showing that the paw on the operated side is withdrawn faster than the paw on the unoperated side, for PWLS is considered an indicator of hyperalgesia. SPET is also a measure of the animals' defensive reaction to a thermal stimulus and so provides another index of hyperalgesia. If this is accepted, then the onset of the hyperalgesia appears to correlate with the initial fiber loss and a predominant loss of large fibers.

DISCUSSION

The central mechanisms for secondary hyperalgesia must involve one or other of the two second order nociceptive neurones, namely the nociceptive specific (NS) or wide-dynamic range (WDR). The former receive high-threshold nociceptive afferents whilst WDR neurones receive convergent inputs from both low-threshold (A) and high threshold (A and C) afferents. The C-fibre polymodal nociceptor is ideally suited to code for the intensity of heat-evoked pain. After injury the peripheral terminals of C-fibre polymodal nociceptor input accounts for an increased output in both nociceptor specific (NS) and wide-dynamic range (WDR) neurones and an increased response to mechanical stimulation. Hyperalgesia to a light touch (mediated by A low threshold mechanoreceptors — LTMs) involves interneurones which have been sensitised to a light touch (6).These interneurones are possibly situated in the superficial dorsal horn or equivalent *superficial subnucleus caudalis laminae.*

Hyperalgesia to heat following damaging stimuli applied to the skin and the continuing pain that develops from inflammation can both be explained by the marked sensitization of C-fibre polymodal nociceptor endings in the central nervous system. Pain is not, however, mediated by C-fibre afferents only (4). A-fibre nociceptors are important for fast nociceptive reflexes and sometimes pain may be mediated by A-fibre low-threshold mechanoreceptors that normally signal non-painful touch sensation.

Neuropathic pain resulting from chronic peripheral nerve injury is often followed by massive alterations in the nNOS expression in the spinal cord too. Recent studies have shown that peripheral nerve lesion induces

degenerative changes mainly in the ipsilateral small and medium neurons which project to laminae I—V. Thus, the major degenerative alterations influence first of all the sensory pathways, throughout the corresponding dorsal rot ganglion's and spinal cord dorsal horn, in the L4—L5 segments (8).

The data suggest, that the neural mechanisms underlying hyperalgesia are based on activation of different modality-specific excitatory amino acid receptor types and subsequently, unique second mesenger systems in the spinal cord. We further contend that there is a pivotal role for neurokinin receptors in the transmission of noxious stimuli, and for the induction of hyperalgesia. Elucidation of the central mechanisms associated with pathological pain in various disorders may one day contribute to novel therapies in these challenging clinical conditions.

REFERENCES

1. Banik, R. K., Kozaki, Y., Sato, J., Gera, L., Mizumura, K., 2001: B 2 receptor-mediated enhanced bradykinin sensitivity of rat cutaneous C-fiber nociceptors during persistent inflammation. *J. Neurophysiol*., 86, 2727—2735.

2. Cabre, A. V., Navaro, X., 2002: Changes in crossed spinal reflexes after peripheral nerve injury and repair. *J. Neurophysiol*., 87, 1763—1771.

3. Craig, A. D., Andrew, D., 2002: Responses of spinothalamic lamina 1 neurons to repeated brief contact heat stimulation in the cat. *J. Neurophysiol*., 87, 1902—1914.

4. Hamalainen, M. M., Gebhart, G. F., Brennan, T. J., 2002: Acute effect of an incision on mechanosensitive afferents in the plantar rat hindpaw. *J. Neurophysiol*., 87, 712—720.

5. Neugebauer, V., Chen, P. S., Willis, W. D., 2000: Groups II and III metabotropic glutamate receptors differentially modulate brief and prolonged nociception in primate STT cells. *J. Neurophysiol*., 84, 2998—3003.

6. Pogatzki, E. M., Gebhart, G. F., Brennan, Y. J., 2002: Characterization of A and C-fibers innervating the plantar rat hindpaw one day after an incision. *J. Neurophysiol*., 87, 731—734.

7. Torsney, C., Fitzgerald, M., 2002: Age depend effects of peripheral inflammation on the electrophysiological properties of neonatal rat dorsal horn neurons. *J. Neurophysiol*., 87, 1311—1317.

8. Xie, Y., Zhang, J., Petersen, M., LaMotte, R.H., 1995: Functional changes in dorsal root ganglion cells after chronic nerve constriction in the rat. *J. Neurophysiol.,* 73, 1811—1820.

9. Zhuo, M., Sengupta, J. N., Gebhart, G. F., 2002: Biphasic modulation of spinal visceral nociceptive transmission from the rostroventral medial medulla in the rat. *J. Neurophysiol*., 87, 2225—2236.

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IMMOBILISATION OF THE BROWN BEAR (*Ursus arctos*) DURING CASTRATION

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ABSTRACT

We describe immobilisation during castration of three brown bears *(Ursus arctos).* The castration was performed with covered *fascia spermatica externa* — a „closed" castration technique by incision at the ventral part of the scrotum, above both testicles, parallel with *the raphe scroti.* Double transfixing ligature was placed to the spermatic cord 1 cm apart, at a distance of about 8 to 10 cm from the testicles. The castration was performed on the request of ZOO in Kavečany-Košice in order to prevent kin mating. It was carried out by means of an immobilisation blowgun using a preparation Large Animal Immobilon® (Reckitt, Colman, England) containing 2.45 mg ethorphine and 10 mg acepromasine in 1 ml volume and an addition of preparation Rometar 2 % inj. a.u.v. (Spofa, Praha, CzR) with xylasine.

Key words: brown bear (*Ursus arctos*); castration; ethorphine; immobilisation

INTRODUCTION

Castration of bears or manipulation with this animal species (capture in free nature, relocation) is not possible without their immobilisation. This intervention is not performed every day therefore the data presented in the specialised literature are not complete. In general, there is relatively little experience with the use of immobilisation and total anaesthesia prior to various surgical interventions in the brown bear. Due to the absence of facial mimic every approach to this animal requires the utmost caution. Even a bear in deep sleep can rise very quickly and attack with full force (2). We were asked by the management of ZOO in Kavečany-Košice to perform castration of three male brown bears (*Ursus arctos*) because of building up new facility for bears where they will live together with a female on a larger area in a free nature. The castration will prevent kin mating.

We describe our experience with castration of three bears and the use of immobilisation preparations Large Animal Immobilon® (Reckitt, Colman, England) and Rometar 2 % inj. a.u.v. (Spofa, Praha, CzR) and an antagonist Large Animal Immobilon – preparation Revivon® (Reckitt, Colman, England).

MATERIAL AND METHODS

We carried out immobilisation and castration of three bears. Two weighed about 180 kg and one about 350 kg. The body weight was seted approximately based on body size from a distance.They were kept in a housing facility with a run and in a room with barred door. The two smaller bears were administered identical doses (1.5 ml) of Large Animal Immobilon together with 0.5 ml Rometar inj. a.u.v. *pro toto*. The larger bear was administered in total 2.5 ml Large Animal Immobilon and 1 ml of Rometar. The immobilisation substances were administered by a shot into forelimb muscles in the scapular region. Revivon® was used as an immobilon antagonist. The immobilisation kit consisted of an immobilisation bullet and a blowgun. During the intervention the animals were monitored continuously. The castration was performed by transfixing ligature of spermatic cords with covered *fascia spermatica externa* – a „closed" castration technique.

RESULTS

After the administration of Large Animal Immobilon and Rometar by means of a blowgun the onset of their action became evident within five minutes, which was

manifested by pronounced sedation of the animals. They assumed a typical sternal position in the corner of a cage with their head bent down and nose resting on the ground between forelimbs. This position is very disadvantageous to the bears as it limits considerably their breathing. Therefore, after the animals assumed this position, we inserted a rod through the barred door and moved their heads. At the same time we tested the response of the treated animal to external stimuli.

Then the animal was put into a prepared net and transferred to a free area where it was fixed by limbs in the dorsal position. The monitoring of animals consisted of observation of their respiration, pulse rate, capillary refill time, and palpebral and ocular reflexes. The pulse was palpated at *arteria lingualis.* It was well palpable, full and regular in all three animals. The heart rates ranged from 40 to 55 beats per minute.

In the second bear, apnoe lasting two minutes was recorded at the beginning of manipulation. Deep inspirations were elicited by pulling the tongue half out and applying Respirot to oral cavity and nose mucosa. The respiratory rate stabilised at one deep inspiration per 40 to 60 seconds. Between these deep inspirations weak inspiration interactions (weak surface inspirations) appeared in this animal which did not suffice to oxygenate completely the animal organism as it was manifested by the dark content of lingual veins. The time of capillary refill time during the immobilisation varied around two seconds. The palpebral reflex was absent and the eye bulbs were fixed in the ventromedial direction.

The bear weighing about 350 kg received 2.50 ml of Large Animal Immobilon pro toto together with 0.5 ml Rometar divided to two shots because the total dose exceeded the volume of one shot. The desired effect was not reached even after 10 minutes. The bear showed weak response to external stimuli and because of that we supplemented the original dose with additional 0.5 ml of Rometar. Within the subsequent 5 minutes we could start the manipulation.

The monitoring of vital functions provided results similar to those observed in the other two animals. The heart rate was a little lower and ranged between 40 and 50 beats per min. No changes in respiration or pulse rates were recorded during the castration. It was performed at deep immobilisation and deep myorelaxation.

The waking up of bears took place already in the new facilities. The mean time of immobilisation and castration together with the transfer of animals to new facilities was 45 min. The animals were roused by preparation Revivon® at a dose double the amount of Large Animal Immobilon®. The first animal was administered the preparation intramuscularly and the animal recovered consciousness in about 30 min. The second bear was administered Revivon® intravenously. It reacted promptly and was able to stand up within 3 min. However, one hour after the administration of Revivon the animal fell asleep again and failed to react to external stimuli. The largest bear was administered the preparation by combining the intravenous and subcutaneous ways, however, during the intravenous administration, the lingual vein cracked. The animal recovered 30 min after administration.

The castration was performed in the dorsal position with the legs bound up. The testicles of bears are located in the scrotum and are palpable and free. When pressure was applied they could not be pushed into the inguinal canals. After cutting off their fur and disinfecting the operational field we used a scalpel to open the scrotum by incision. The testicles were pulled out into the operation field. The spermatic cords were covered by adipose tissue that was removed using a blunt edge. Then two ligatures (Dexon) were placed at an 8—10 cm distance from the testicles, approx. 1 cm apart, and the testicles were resected. The castration wounds were left open and were treated locally by antibiotic lavage. The castration was followed by total administration of antibiotic Penicillin Biotika Depot a.u.v. (Biotika, SR), antiflogistic Finadyne sol. a.u.v. (Schering-Plough Animal Health, France), antitetanus serum Clotean Inj. a.u.v. (Bioveta, CzR), and antihistaminic Ancesol a.u.v. (Richter Pharma, Austria).

No postoperative complications were observed in the bears. The monitored animals showed no signs of pain. Final inspection of all animals revealed no complications.

DISCUSSION

Several combinations of preparations are recommended for immobilisation of the brown bear. We selected the combination of ethorphine and acepromasine in a preparation Large Animal Immobilon®, together with an addition of xylasine in the preparation Rometar 2 % inj. a.u.v. The dose for the brown bear with this combination is 16.82 µg. kg^{-1} ethorphine (1). The addition of xylasine ranges from 0.09 to 0.18 $mg.kg^{-1}$. This combination is suitable even when very painful operation is performed (3).

The advantages of Immobilon include a high effectiveness of action on animals, rapidity of onset, and small-administered volume while the disadvantages are necessity of using protective devices, high toxicity and the related risk to veterinarians (3). Its disadvantages to animals are also considerable. The most important are the suppression of the respiratory centre, potential excitations, particularly with insufficient doses, convulsions, muscle tremor, bradycardia, hyperthermia, enterohepatal circulation, and others (4). The negative effects depend on both the dose used and the individual itself.

The most serious disadvantage is the suppression of ventilation, which together with enterohepatal circulation, results in the persistence of its effect even for 7 to 8 hours. Because of that, when this preparation is used, a specific antidotum is administered after the intervention as soon as possible (4). Diprenorphin hydrochloride in the preparation Revivon® is an antagonist of ethorphine which reliably reverses its effects. It is administered

intramuscularly, subcutaneously, or intravenously in a volume twice that of Large Animal Immobilon® (1). As an opium derivative belonging to the group of agonists-antagonists it has an analgesic action (4) so it guarantees postoperative analgesis.

One of the main advantages of Large Animal Immobilon is its small volume needed for preparation of a shot so it can be administered simply by means of a blowgun. The immobilisation induced by this preparation in animals is deep and more than sufficient. No protective responses were observed during the intervention so the castration was performed relatively quickly and without complications. With regard to relatively pronounced breathing suppression, the intervention should be performed in the shortest possible time and after its completing the animal should be administered the antagonistic, Large Animal Immobilon. The animal reacts quickly when the antagonist is administered intravenously, however its effect is not as lasting as that observed after its i.m. administration. However, the animal returns faster to its normal state.

The success of the operation and its rapid performance depends on thorough preparation of all instruments and materials. Good organisation and sufficient number of helpers is important for smooth operation.

CONCLUSION

The combination of preparations Large Animal Immobilon® (Reckitt, Colman, England) and Rometar 2 % inj. a.u.v. (Spofa, CzR) ensured sufficiently long immobilisation of all three brown bears subjected to castration. During the entire operation none of them showed response to painful stimuli or resistance to manipulation. The onset of action of the substance and revival of the animals was rapid and calm without excitation. A marked disadvantage of the immobilisation method used was suppression of breathing and our inability to compensate it significantly.

The administration of antagonist — Revivon® (Reckitt, Colman, England) ensured the rapid recovery of animals, particularly after its i.v. administration. The intervention should be performed rapidly and the animal should remain in the immobilised state only for the required time. This method of immobilisation appears suitable for the castration of brown bear males but also for other surgical interventions.

REFERENCES

1. Gatesman, T., Wiesner, H., 1982: Immobilization of polar *(Thalarctos maritimus)* and brown *(Ursus arctos)* bear using etorphine and xylazine. *J. Zoo Anim. Med.,* 15, 11—18.

2. Hatlapa, H. H. M., Wiesner, H., Eriksen, E., Fritsch, R., Merkel-Gottlieb, A., 1982: *Die Praxis der Wieldtier-immobilisation.* Hamburg (Germany, F. R.) Parey, 96 pp.

3. Hess, L., Dvořáček, V., Svobodník, J., 1988: *Anesthesia and Immobilization of the Zoo Animals* (In Czech). Avicenum Prague, 256 pp.

4. Nielsen, L., 1996: Chemical immobilization of freeranging terrestrial mammals. In **Thurman *et al.*:** *Lumb & Jones' Veterinary Anesthesia (Third edition). Williams and Wilkins, Baltimore, 736—764.*

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THE COMPARISON OF HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS IN NATURAL CANINE DISTEMPER VIRUS INFECTION

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ABSTRACT

From June 2000 to August 2001, nine dead or euthanised sick dogs were submitted to our laboratory for necropsy. Gastrointestinal, respiratory and neurological signs were prominent in all the affected dogs. The most striking features in the necropsy were gastroenteritis and pneumonia. Catarrhal gastroenteritis, bronchitis, bronchiolitis and interstitial pneumonia in the lungs, nonpurulent demyelinating encephalitis, lymphocytic necrosis in the spleen and other lymphoid tissues were clear under microscopic examination. Intracytoplasmic and intranuclear inclusion bodies were seen in the stomach, intestine, spleen, mediastinal lymph nodes, lung, cerebrum, cerebellum, pelvic renum epithelium, urinary bladder epithelium and the salivary gland in haematoxylin and eosin staining. The stomach, intestine, liver, lung, cerebrum, cerebellum, plexus choroideus, heart, adrenal gland, pancreas, spleen, mediastinal and the mesenteric lymph nodes, kidney, urinary bladder, and the salivary gland were found to be canine distemper virus (CDV) positive following the application of an immunohistochemical staining technique. There was no immunostaining in the skin and eye. The results suggest that although the inclusion bodies in the tissues are important diagnostic criteria for confirming CDV infection, the immunohistochemical detection of the CDV antigen is always the most important criteria.

Key words: CDV; histopathology; immunohistochemistry

INTRODUCTION

Canine distemper virus, a morbillivirus of the Paramyxoviridae, causes a multisystemic disease of dogs and other carnivores (6, 12). CDV possesses a wide host spectrum and numerous different species (10). In spite of the development of effective vaccines, the disease remains endemic in most parts of the world.

The early stages of infection with canine distemper virus involve the gastrointestinal and upper respiratory tracts and are characterized by diarrhoea and ocular and nasal discharges. In some cases neurological signs occur at a later stage, and distemper is regarded as the commonest cause of neurological diseases, including ataxia and paresis/paraplegia in dogs. In microscopic sections, characteristic cytoplasmic, and intranuclear inclusion bodies are often seen in cells present in the exsudate. The immunofluorescence technique may be used to demonstrate that these inclusions contain viral antigen. The stomach and intestines may contain large numbers of cytoplasmic and some intranuclear inclusions in the lining epithelium (1, 2, 13, 10, 12).

Apart from these cell inclusions, few lesions were observed. Immunohistochemical techniques allow the demonstration of infectious antigens in tissue sections and CDV antigen has been demonstrated in several tissues including the brain (3, 11, 8, 9, 6, 16, 5).

The purpose of this report is to compare the histopathological and immunohistochemical findings in naturally occurring CDV infection.

MATERIAL AND METHODS

From June 2000 to August 2001, nine animals were examined, all of which were suspected to have CDV infection and had either died (No. 1—4) or been humanely killed (No. 5—9) at the moribund stage. The age, sex and breed details of the animals and clinical findings are summarized in Table 1. These animals were submitted to our laboratory for necropsy. Samples were collected from the lung, liver, cerebrum, cerebellum, heart, stomach, intestine, adrenal gland, pancreas,

spleen, mediastinal and mesenteric lymph nodes, kidney, urinary bladder, skin, eye, and the salivary gland from all dogs. Samples were fixed in 10 % buffered formalin and embedded in paraffin wax. For light microscopy, five μm thick sections were stained with haematoxylin and eosin.

An immunohistochemical examination was performed for all of the sections. CDV antigen was demonstrated by immunohistochemistry using the streptavidin-biotin-peroxidase method. The sections were cut at five μm and dewaxed in xylene and rehydrated in alcohol. All steps unless indicated otherwise were done at room temperature in a dark humidified chamber. Tris buffer solution (TBS, pH 7.4) was used for each washing step. Monoclonal antibody against CDV and universal LSAB2 horse radish peroxidase (HRP) kit (Dako, Glostrup, Denmark) were used for the demonstration of CDV antigen. The sections were dried overnight at 37 °C and dewaxed in two changes of xylene for ten minutes each, rehydrated in 100 %, 95 % and 70 % alcohol and placed in distilled water for ten minutes.

All steps were performed at room temperature and in a humidity chamber. Two changes of tris-buffer (pH 7.4) were used for washing. Endogenous peroxidase was blocked with 3 % hydrogen peroxide in distilled water for five minutes. The sections were boiled with antigen retrieval solution (Dako, Glostrup, Denmark, Cat. No. S 1699) for forty minutes, and were blocked with protein blocking agent (Dako, Glostrup, Denmark, Cat No. X 0909) for five minutes.

The sections were then incubated with 1/128 diluted in PBS (pH 7.4) mouse anti-canine distemper virus antibody for fifty minutes, anti-mouse biotinylated polyvalent secondary antibody in PBS for ten minutes, streptavidin-peroxidase enzyme for ten minutes, amino ethyl carbazole (AEC) in hydrogen peroxide chromogen for ten to fifteen minutes (controlled by visual observation with a microscope) and rinsed with distilled water. Sections were counterstained with Mayer's haematoxylin for one to two minutes, rinsed with distilled water and mounted with aqueous mounting medium.

In each batch of sections stained known positive and negative control tissues were included.

RESULTS

Clinical findings

The clinical findings of all the dogs are summarized in Table 1.

The affected dogs showed gastrointestinal, respiratory and neurological signs. Diphasic fever were (39.5—41 °C) was found in all the infected dogs. There were anorexia, dehydration, vomiting, diarrhoea ranging from catarrhal to haemorrhagic and weight loss in many dogs. Respiratory findings ranged from serous to mucopurulent naso-ocular discharge and coughing. Ataxia, convulsions, tic, seizures, incoordination and opisthotonus developed as neurological signs in most affected dogs.

Macroscopic findings

The gastrointestinal lesions ranged from catarrhal to haemorrhagic. Severe pneumonia was the main necropsy finding in some of the dogs. These lungs did not collapse. Dark red consolidation areas were seen in all lobes. There was emphysema in the caudal lobes and interlobular areas. Mediastinal lymph nodes were enlarged. There was mucopurulent exsudate in the trachea, bronchi and bronchioles.

Microscopic findings

Stomach lesions: Many intranuclear and intracytoplasmic inclusion bodies were seen especially in the deep layer of the *lamina propria. The chief cells were important for inclusion bodies.*

Lung lesions: There was broncho-interstitial pneumonia in all the dogs. The pneumonia was initially interstitial but later bronchopneumonia developed. The

Table 1. Details of the animals for age, sex, breed and clinical findings

Case No.	Age	Sex	Breed	Clinical findings
1	1 year	female	Turkish sheep dog	Vomiting, haemorrhagic diarrhoea, weight loss, coughing
2	5 months	female	Crossbreed	Catarrhal diarrhoea, anorexia, weight loss
3	6 months	male	Collie	Vomiting, dehydration, weight loss, coughing, naso-ocular discharge, tic, incoordination
4	3 months	male	Terrier	Haemorrhagic diarrhoea, weight loss, convulsions, opisthotonus, seizures
5	7 months	male	Crossbreed	Nasal discharge, opisthotonus
6	5 months	female	Crossbreed	Haemorrhagic diarrhoea, weight loss, naso-ocular discharge, tic
7	1 year	male	Dobermann	Coughing, opisthotonus, tic, ataxia
8	3 months	female	Terrier	Coughing, tic, convulsions, ataxia
9	1 year	female	Crossbreed	Catarrhal diarrhoea, naso-ocular discharge, seizures, opisthotonus

Table 2. The distribution of inclusion bodies in various tissues by haematoxylin-eosin staining

Case No.	stom.	int.	liv.	lung	kid.	spl.	adren. gl.	sal. gl.	panc.	lym. node	cer.	cere-bell.	eye	uri. blad.	skin	heart
1	+++	+	-	++	-	+	-	-	-	-	-	-	-	+	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	-	-	++	-	-	-	-	-	-	++	+++	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
5	++	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-
6	-	-	-	-	+	-	-	-	-	-	-	+	-	+++	-	-
7	++	-	-	+	+	-	-	-	-	-	-	+++	-	+	-	-
8	++	+	-	+	+	+	-	-	-	+	-	++	-	+	-	-
9	+++	+	-	++	+	+	-	+	-	+	-	+++	-	+	-	-

Legend: stom — stomach; int — intestine; liv — liver; kid — kidney; spl — spleen; sal. gl — salivary gland; panc — pancreas; lym. node — lymph node; cer — cerebrum; cerebell — cerebellum; uri. blad — urinary bladder

+ — mild; ++ — moderate; +++ — severe; - — no lesions

Table 3. The distribution of CDV in various tissues by immunohistochemistry

Case No.	stom.	int.	liv.	lung	kid.	spl.	adren. gl.	sal. gl.	panc.	lym. node	cer.	cere-bell.	eye	uri. blad.	skin	heart
1	+++	+	++	+++	+	+++	-	+	-	-	-	-	-	+++	-	+
2	-	+++	-	-	+	-	-	-	++	+	+++	-	-	-	-	+
3	++	+	-	+++	+	-	++	-	-	-	+++	+++	-	-	-	-
4	-	+++	+	+	-	-	-	+	+	-	+	+++	-	-	-	-
5	++	++	-	++	++	++	++	+	-	++	-	++	-	-	-	-
6	+++	+++	-	+++	+++	+++	-	++	+	-	+++	+++	-	+++	-	-
7	++	++	-	++	++	++	-	+	+	+	++	+++	-	++	-	-
8	++	+	-	++	+	+	-	-	-	+	+	++	-	+	-	+
9	+++	++	+	+++	+	++	+	++	+	++	++	++	-	+	-	-

Legend: stom — stomach; int — intestine; liv — liver; kid — kidney; spl — spleen; sal. gl — salivary gland; panc — pancreas; lym. node — lymph node; cer — cerebrum; cerebell — cerebellum; uri. blad — urinary bladder

+ — mild; ++ — moderate; +++ — severe; - — no lesions

proliferation of type-II pneumocytes and giant cells was prominent. Lymphocytes, leukocytes and macrophages were marked in interstitial areas. There were intracytoplasmic inclusion bodies in bronchial and bronchiolar epithelial cells and alveolar macrophages. In some areas bronchial and bronchiolar epithelial cells were necrotic. Bronchiolar lumina filled with serofibrinous fluid, macrophages and giant cells. There was emphysema and oedema in the interstitial areas.

Brain lesions: Demyelination and perivascular mononuclear cuffing were marked in the substantia alba of the cerebellum. Demyelination was ranking from moderate to severe. Focal gliosis and the proliferation of astrocytes and microglia were common. Gemastocytic astrocytes and giant cells were seen in demyelinated areas. There were many intracytoplasmic and intranuclear inclusion bodies in astrocytes, microglia, and ependymal cells. The proliferation of capillaries was prominent. There were many necrotic neurons in the cortex. Mononuclear cell infiltrations were seen in meninges.

The spleen and lymphoid tissues: The degeneration and necrosis of the lymphocytes in the lymphoid follicles were prominent in both the spleen and the other lymphoid tissues.

The urinary bladder and renal pelvis le-si ons: Intranuclear and intracytoplasmic inclusion bodies were seen in the transitional epithelial cells of the urinary bladder and renal pelvis.

The distribution of intracytoplasmic and intranuclear inclusion bodies in various tissues stained with haematoxylin-eosin are shown in Table 2.

Immunohistochemical findings

The positive characteristic staining with the

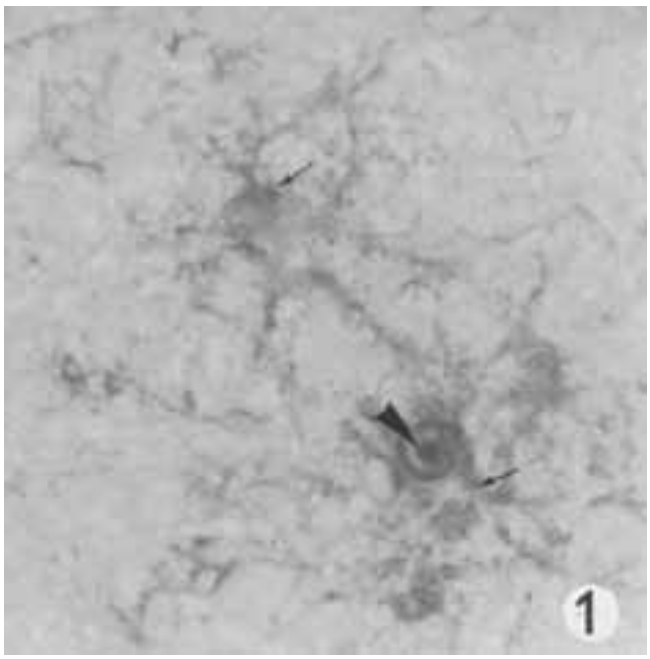

Fig. 1. A section of the cerebellum from a dog infected with CDV, showing the immunolocalization of the viral antigens in the cytoplasms of the fibrous astrocytes *(arrows)* and the intranuclear inclusion body in the astrocyte *(arrow head)* by AEC chromogen, streptavidin-peroxidase (640×)

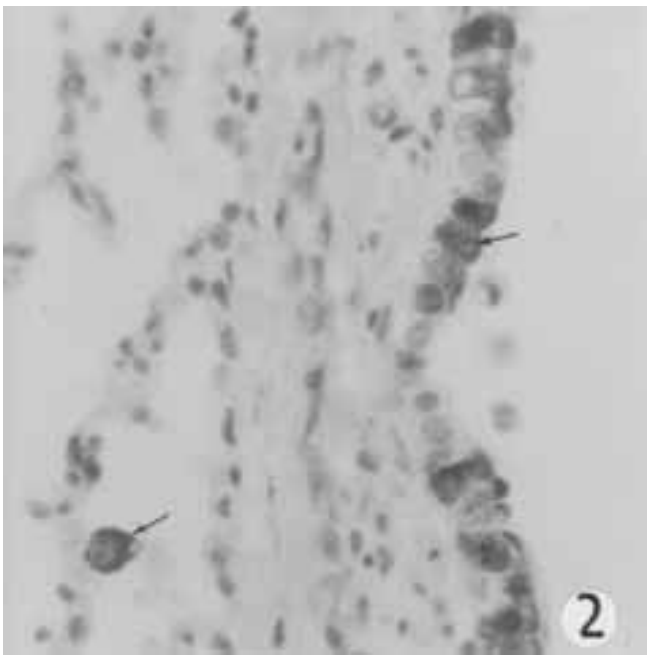

Fig. 2. A section of lung from a dog infected with CDV, showing the immunolocalization of the viral antigens in both bronchiolar epithelium and alveolar macrophage *(arrows)* by AEC chromogen, streptavidin-peroxidase (320×)

immunoperoxidase technique were seen as red colour. The distribution of CDV in various tissues by immunohistochemistry are shown (Table 3). Many organs were stained as positive. Cerebrum, cerebellum (Fig. 1), plexus choroideus, stomach, intestine, lung (Fig. 2), liver, heart, adrenal gland, pancreas, salivary gland, kidney, urinary bladder, spleen, mediastinal and mesenteric lymph nodes, were marked seen as positive. There were many intracytoplasmic and intranuclear inclusion bodies in most immunostained tissues.

DISCUSSION

In this study, histopathologically the dogs showed numerous inclusion bodies in their lungs, stomachs, intestines, renal pelvises, urinary bladders, cerebrums, cerebellums, spleens, lymph nodes and salivary glands. The histopathological diagnosis was supported by the immunohistochemical detection of the CDV antigen in most of the tissues.

In our study, CDV antigens were more prominent and more easily found than the inclusion bodies in tissue sections (13). Although the inclusion bodies are histopathologically important diagnostic criteria for confirming CDV infection, the immunohistochemical demonstration of the CDV antigen is always the most important criterion. Furthermore, immunohistochemical examination showed the virus in the some of the tissues, such as the liver, pancreas, salivary gland, adrenal glands, despite the absence of obvious histopathological lesions.

Streptavidin-biotin-peroxidase immunostaining method is the more efficient method than the avidin-biotin-peroxidase complex (ABC) method or peroxidase-antiperoxidase method for demonstrating CDV antigen (3, 7). In our study, CDV antigens were easily found in tissues by the streptavidin-biotin-peroxidase method and the sections were also boiled with the target retrieval antigen unmasking solution. When the tissues were treated with this solution, the CDV antigen was more prominent in the sections.

In CDV infection, although the inclusion bodies can be seen intranuclearly in neurons and astrocytes in the central nervous system (14, 4), in this study, the inclusion bodies were only seen in both astrocytes.

Appel (1) suggested that the range of clinical signs are affected by factors such as the host's age, natural resistance to the virus and secondary infections. Vaccination is also an important factor for the disease.

In this study, the necrosis of lymphoid follicles of the spleen and lymph nodes were seen. This finding suggested thought that the CDV replicates in the lymphoid tissues and spreads to other tissues by infected lymphocytes (1, 15).

REFERENCES

1. Appel, M. J. G., 1969: Pathogenesis of canine distemper. *Am. J. Vet. Res.,* 30, 1167—1182.

2. Appel, M. J. G., 1987: Canine distemper virus. In **Horzinek, M. C. (ed.):** *Virus Infections of Carnivores,* Vol. 1. Elsevier, Amsterdam, pp. 133—159.

3. Axthelm, M. K., Krakowka, S., 1986: Immunocytochemical methods for demonstrating canine distemper virus antigen in aldehyde-fixed paraffin-embedded tissue. *J. Vir. Meth.,* 13, 215—229.

4. Gröne, A., Alldinger, S., Baumgartner, W., 2000: Interleukin-1β, -6, -12 and tumor necrosis factor-α expression in brains of dogs with canine distemper virus infection. *J. Neuroimmunol.,* 110, 20—30.

5. Headley, S. A., Soares, I. C., Graça, D. L., 2001: Glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes in dogs infected with canine distemper virus. *J. Comp. Path.,* 125, 90—97.

6. Iwatsuki, K., Okita, M., Ochikubo, F., Gemma, T., Shin, Y. S., Miyashita, N., Mikami, T., Kai, C., 1995: Immunohistochemical analysis of the lymphoid organs of dogs naturally infected with canine distemper virus. *J. Comp. Path.,* 113, 185—190.

7. Kennedy, S., Smyth, J. A., Cush, P. F., Duignan, P., Platten, M., McCullough, S. J., Allan, G. M., 1989: Histopathologic and immunocytochemical studies of distemper in seals. *Vet. Pathol.,* 26, 97—103.

8. Kennedy, S., Smyth, J. A., Cush, P. F., McAliskey, M., McCullough, S. J., Rima, B. K., 1991: Histopathologic and immunocytochemical studies of distemper in harbor porpoises. *Vet. Pathol.,* 28, 1—7.

9. Mitchell, W. J., Summers, B. A., Appel, M. J. G., 1991: Viral expression in experimental canine distemper demyelinating encephalitis. *J. Comp. Path.,* 104, 77—87.

10. Moll, P. V., Alldinger, S., Baumgartner, W., Adami, M., 1995: Distemper in wild carnivores: An epidemiological, histological and immunocytochemical study. *Vet. Microbiol.,* 44, 193—199.

11. Mutinelli, F., Vandevelde, M., Griot, C., Richard, A., 1988: Astrocytic infection in canine distemper virus-induced demyelination. *Acta Neuropathol.,* 77, 333—335.

12. Okita, M., Yanai, T., Ochikuboţ, F., Gemma, T., Mori, T., Maseki, T., Yamanouchi, K., Mikami, T., Kai, C., 1997: Histopathological features of canine distemper recently observed in Japan. *J. Comp. Path.,* 116, 403—408.

13. Palmer, D. G., Huxtable, C. R. R., Thomas, J. B., 1990: Immunohistochemical demonstration of canine distemper virus antigen as an aid in the diagnosis of canine distemper encephalomyelitis. *Res. Vet. Sci.,* 49, 177—181.

14. Summers, B. A., Cummings, J. F., de Lahunta, A., 1995: Inflammatory diseases of the central nervous system. In **Duncan, L. (ed.):** *Veterinary Neuropathology.* Mosby Year Book, USA, pp. 102—110.

15. Summers, B. A., Griesen, H. A., Appel, M. J. G., 1979: Early events in canine distemper demyelinating encephalomyelitis. *Acta Neuropathol.,* 46, 1—10.

16. Wünschmann, A., Alldinger, S., Kremmer, E., Baumgartner, W., 1999: Identification of CD4+ and CD8+ T cell subsets and B cells in the brain of dogs with spontaneous acute-, subacute-, and chronic-demyelinating distemper encephalitis. *Vet. Immunol. Immunopathol.,* 67, 101—116.

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ACID-BASE BALANCE ASSESSMENT DURING EXERCISE IN THE SHOW JUMPING HORSE

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ABSTRACT

The authors have studied the pattern of several blood gas parameters during physical exercise in the show jumping horse. Therefore changes of the acid base balance and of blood gas parameters were investigated in five healthy horses competing in an official show jumping event. To define the parameters of the blood gas pattern, blood samples were collected at rest, after warm-up, after the trial, and after a thirty and sixty minute period of recovery. The following parameters were determined: pH, CO_2 partial pressure (pCO_2), oxygen partial pressure (pO_2), oxygen saturation percentage (SO_2%), haematocrit (Hct), haemoglobin (Hb), sodium (Na^+), base excess of extracellular fluid (BE-ecf), base excess of the blood (BE-b), standard bicarbonate concentration (SBC), bicarbonate level (HCO_3^-), total carbon dioxide (TCO_2), alveolar oxygen (A), oxygen capacity (O_2Cap) and oxygen content (O_2Ct). This task was carried out using a selective ions analyser and elaborating experimental data by means of a Student *t*-test for paired data. The following parameters showed no statistically significant differences: pH, pCO_2, SO_2 %, HCO_3^-, TCO_2, A, and SBC. On the other hand the following parameters showed statistically significant differences: BE-ecf, BE-b, Hct, Hb, O_2Cap, O_2Ct and pO_2. The results obtained provide a valuable contribution for the assessment of the horse's performance level and for the monitoring of training program effectiveness.

Key words: athletic horse; blood gas parameters; exercise physiology

INTRODUCTION

In equine sport medical investigations have been carried out with the purpose of defining reliable parameters for the horse's performance assessment. Many studies have been carried out with the purpose of underlining the pattern of some haematochemical and haematological parameters during training and physical exercise in the jumping horse. Haematology has long been used to identify correlations with training or to predict potential racing performance.

However doubts have been expressed on haematological reliability in predicting performance or in assessing improved fitness (19). More recently plasma biochemical and blood gas values have been examined to determine the effect of exercise on various physiological processes. In order to evaluate the response to training programs, many performance profiles, including haematological, plasma biochemical analysis, muscle biopsy and exercise tests, are usually carried out (6). As a matter of fact, exercise is correlated with changes in acid-base status as a consequence of muscular anaerobic metabolism (1). Electrolytes play a vital role in supporting several biological functions involved in strenuous exercise. These functions include nerve conduction and muscle contraction, muscle blood flow, plasma or water volume homeostasis, cellular metabolism, acid-base balance, cardiovascular functions, blood clotting and skeletal growth (8).

The present study aims at analysing the variations of the acid-base status in the venous blood of athlete horses before and after a show jumping trial, as well as at rest and after recovery.

Recordings have been made of venous blood as many parameters including HCO_3^-, TCO_2, PCV, Na^+, K^+ do not show statistical differences with the same parameters obtained for arterial blood (1, 3, 18, 23, 24). Furthermore, the venous blood TCO_2 value is used to evaluate sodium bicarbonate adminis-

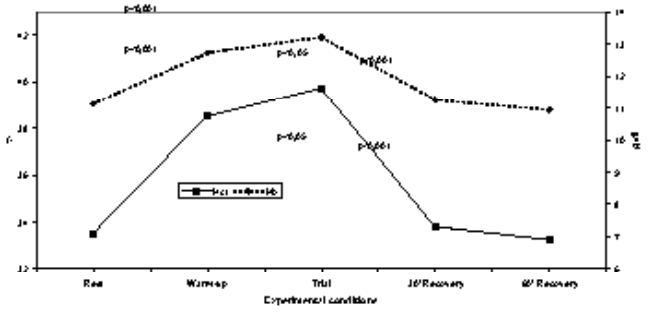

Fig. 1. Haematocrit (%) and haemoglobin (g.dl^{-1}) pattern in 5 jumping horses under different experimental conditions

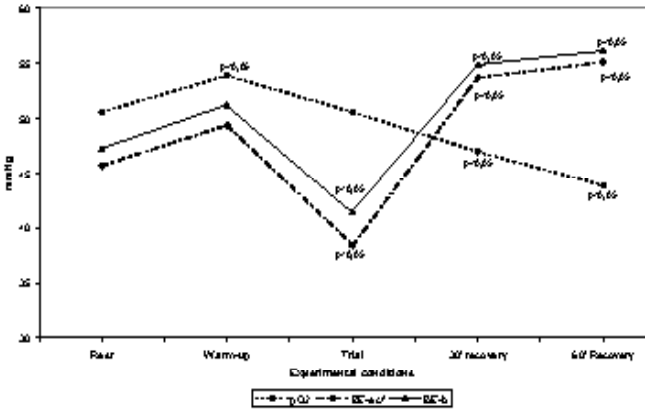

Fig. 3. Oxygen partial pressure (mmHg), base excess in the extra cellular fluid (mmol.l^{-1}) and base excess in the blood (mmol.l^{-1}) pattern in 5 jumping horses under different experimental conditions

tration as a doping substance (milkshake). Indeed bicarbonate administration enhances the blood and muscular extracellular fluid buffering function (11, 13, 7, 12, 9). The consequent faster removal of hydrogen ions by active muscles delays the onset of fatigue after highly intensive exercise (14, 17).

MATERIALS AND METHODS

The study was carried out on five "Sella Italiana" females jumpers aged 13 years. All horses under examination were clinically healthy. They were fed three times a day on hay and concentrates (morning — hay, midday — hay, evening — hay and concentrate).

The tested horses underwent the following program: ten minute walk warm-up, fifteen minutes trot and five minutes gallop followed immediately by a show jumping course made from eight obstacles (height: 90 m and 110 cm; course length: 300 m).

Blood was drawn by means of an external jugular venipuncture using a blood sampling kit for blood-gas-analysis (1 cc plastic syringe containing approx. 15 units of lyophilised lithium heparin derived from porcine intestinal mucosa). Samples were drawn at rest, after warm-up, after the trial (the competition) and thirty and sixty minutes after the end of the trial. By means of a selective ions analyser (Stat Profile pHOx Analyser — Nova Biomedical), the following parameters were evaluated: pH, CO_2

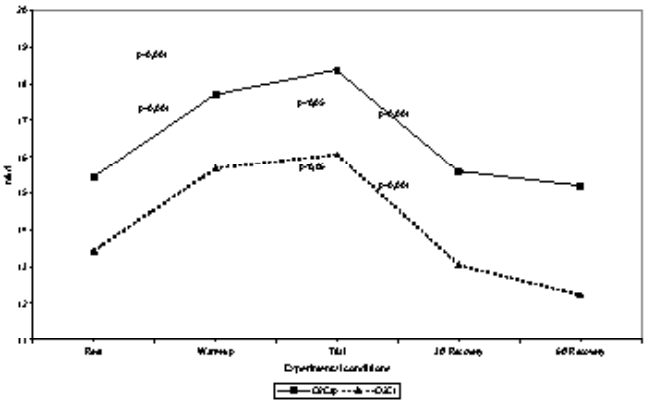

Fig. 2. Oxygen capacity (ml.dl^{-1}) and oxygen content (ml.dl^{-1}) pattern in 5 jumping horses under different experimental conditions

partial pressure (pCO_2), oxygen partial pressure (pO_2), oxygen saturation percentage (SO_2%), haematocrit (Hct), haemoglobin (Hb), sodium (Na^+), base excess of extra-cellular fluid (BE-ecf), base excess of the blood (BE-b), standard bicarbonate concentration (SBC), bicarbonate level (HCO_3^-), total carbon dioxide (TCO_2), alveolar oxygen (A), oxygen capacity (O_2Cap) and oxygen content (O_2Ct).

Measurements were carried out as recommended by the National Committee of Blood Laboratory Standards (*Considerations in the Simultaneous Measurement of Blood Gases, Electrolytes and Related Analytes in Whole Blood; Proposed Guidelines*). For each test the analyser operating temperature was set according to the horse rectal temperature recorded during sampling.

Since the intragroup variance of the obtained data for the five subjects was not significant, it was possible to elaborate the mean values statistically. The Student *t*-test for paired data was applied to determine any statistical significance between parameters values recorded at rest and after warm-up and between parameters values recorded after warm-up and after the other experimental conditions.

RESULTS

Tab. 1 shows the mean values of the parameters under investigation (pH, pCO_2, pO_2, SO_2%, PCV, Hb, Na^+, BE-ecf, BE-b, SBC, HCO_3^-, TCO_2, A, O_2Cap, O_2Ct), expressed in conventional measurement units with standard deviations.

The following parameters have shown no statistically significant differences: pH, pCO_2, SO_2%, HCO_3^-, TCO_2, A and SBC.

BE-ecf and BE-b showed statistically significant differences between the values obtained after warm-up and after the jumping trial ($P<0.05$), and between the values obtained after the trial and after a thirty minute ($P<0.05$) and a sixty minute ($P<0.05$) recovery period (Fig. 3).

Statistically significant differences were also observed for Hct, Hb (Fig. 1), O_2Cap and O_2Ct (Fig. 2) comparing the values obtained at rest with those recorded after

Table 1. Blood gas parameters mean values expressed in conventional measurement units, with relative standard deviations

PARAMETERS	EXPERIMENTAL CONDITIONS				
	REST	WARM-UP	TRIAL	30'RECOVERY	60'RECOVERY
pH	7.46 ± 0.01	7.47 ± 0.01	7.45 ± 0.03	7.46 ± 0.02	7.46 ± 0.02
pCO_2 (mmHg)	41.95 ± 2.52	41.00 ± 2.84	41.98 ± 3.71	43.20 ± 2.19	43.27 ± 3.13
PO_2 (mmHg)	50.55 ± 2.54	53.88 ± 2.54	50.55 ± 4.74	46.96 ± 1.83	43.85 ± 1.69
SO_2 (%)	86.45 ± 1.68	87.70 ± 0.89	84.78 ± 7.03	82.66 ± 2.42	79.88 ± 3.12
Hct (%)	33.50 ± 2.06	38.50 ± 1.50	39.72 ± 2.47	33.80 ± 2.59	33.25 ± 1.64
Hb ($g.dl^{-1}$)	11.12 ± 0.63	12.72 ± 0.44	13.21 ± 0.78	11.24 ± 0.80	10.92 ± 0.46
BE-ecf($mmol.^{-1}$)	5.61 ± 0.77	6.23 ± 0.82	4.40 ± 1.52	6.94 ± 0.98	7.18 ± 0.72
BE-b ($mmol.^{-1}$)	5.87 ± 0.56	6.53 ± 0.58	4.90 ± 1.27	7.14 ± 0.90	7.35 ± 0.54
SBC ($mmol.^{-1}$)	30.27 ± 1.82	30.70 ± 1.35	29.12 ± 2.11	30.64 ± 0.86	30.80 ± 0.51
HCO_3^-($mmol.^{-1}$)	30.28 ± 2.12	30.23 ± 1.76	28.98 ± 2.48	30.86 ± 0.79	31.08 ± 0.92
TCO_2 ($mmol.^{-1}$)	31.55 ± 2.19	31.45 ± 1.84	30.20 ± 2.55	32.12 ± 0.83	32.35 ± 1.00
A (mmHg)	99.95 ± 3.05	100.63 ± 3.34	99.28 ± 4.41	98.28 ± 2.93	98.33 ± 3.79
O_2Cap ($ml.dl^{-1}$)	15.43 ± 0.86	17.70 ± 0.60	18.38 ± 1.09	15.60 ± 1.13	15.20 ± 0.65
O_2Ct ($ml.dl^{-1}$)	13.43 ± 0.91	15.68 ± 0.40	16.06 ± 1.09	13.04 ± 1.03	12.23 ± 0.31

warm-up ($P<0.001$), and between trial values with those recorded thirty minutes ($P<0.05$) and sixty minutes after the end of the trial ($P<0.001$).

Finally, from the statistical examination of pO_2 (Fig. 3), statistically significant differences were observed between data obtained at rest with those recorded after warm-up ($P<0.05$) and between data obtained after the trial with those recorded after a thirty and a sixty minute recovery period ($P<0.05$).

EVALUATION OF RESULTS, DISCUSSION AND CONCLUSION

The first conclusion which can be drawn from this study is that resting values of the parameters under investigation are within the range of "normal values", as reported by specialised literature on the horse (17, 4).

These values are a useful starting point and can be compared to values recorded after the trial in order to demonstrate the influence of physical exercise on the above parameters.

The analysis of the obtained results shows that blood pH did not reveal statistically significant differences. This is due to the activation of the following compensation mechanisms: 1) a chemical buffer from extracellular and intracellular buffers; 2) blood CO_2 partial pressure control through alveolar ventilation changes; 3) bicarbonate plasmatic concentration control through variations of hydrogen ions (H^+) renal excretion (20). Indeed the regulation of H^+ concentration is essential for normal cellular functions, since hydrogen ions react heavily with proteins.

As for pO_2, a progressive increase during warm-up and a gradual decrease up to sixty minutes after the end of the trial were noticed. The initial increase could be correlated with increased ventilation. The subsequent decrease could be the consequence of pO_2 reduction in the venous area due to tissue uptake and exchange with carbon dioxide (13).

The significant haematocrit (Hct) and haemoglobin increase observed after warm-up and after the trial is primarily due to the mobilisation of splenic reserve (red cells), but also to the decrease of plasmatic volume (16), as a result of fluids shifting from the vascular compartment to interstitial compartment, and sweating (15). This shifting of fluids from the vascular compartment to interstitial and intracellular compartments is the result of osmotic and hydrostatic processes (10, 21). The release of the splenic reserve can dramatically increase the oxygen-carrying capacity of the blood thus enhancing the horse's ability to withstand long periods of maximal exercise (22). An increase in haemoglobin concentration and erythrocytes enhances the ability to buffer the acids produced by metabolism and facilitates CO_2 carrying through the "hydrochloric exchange" system (5). Clearly also the O_2Ct and O_2Cap parameters follow the haemoglobin pattern since the percentage of oxygen dissolved in venous blood is not significant.

BE-ecf and BE-b values showed a significant decrease after warm-up due to bases and buffer bicarbonate consumption, thus revealing the activation of the buffer systems (6). On the other hand, immediately after the trial there was a significant BE-ecf and BE-b increase that could be correlated to lactate excess with consequent inadequate buffering (2).

On the base of the results obtained, it can be stated that the monitoring of blood gas parameters during training and competition provides useful indications about the efficiency of the training program and on the athletic horse's level of performance.

REFERENCES

1. Adroguè, H. J., Rashad, M. N., Gorin, A. B., Yacoub, J., Madias, N., 1989: Assessing acid-base status in circulatory failure. *New Engl. J. Med.,* 320—20, 1312.

2. Aguilera-Tejero, E., Bas, S., Estepa, J. C., Lòpez, I., Mayer-Valor, R., Rodriguez, M., 2001: Acid-base balance after exercise in show jumpers. *CESMAS,* 43—45.

3. Bleich, H. L., 1989: The clinical implications of venous carbon dioxide tension. *New Engl. J. Med.,* 320—20, 1345.

4. Carlson, G. P., 1989: In **Kaneko, J. J. (ed.), 1989:** *Fluid, Electrolyte, and Acid-Base Balance. Clinical Biochemistry of Domestic Animals.* Academic Press, 543—575.

5. Carlson, G. P., 1995: Interrelationships between fluid, electrolyte and acid-base balance during maximal exercise. *Equine Vet. J. Suppl.,* 18, 261—265.

6. Corazza, M., Buonaccorsi, A., Ercolini, F., Barsaglini, M., 1995: Valutazione dello stato di forma del cavallo atleta: lattacidemia e parametri emogasanalitici. *Rivista Ufficiale SIDI,* 1, (1).

7. Freestone, J. F., Carlson, G. P., Harrold, D. R., Church, G., 1989: Furosemide and sodium bicarbonate-induced alkalosis in the horse and response to oral KCl or NaCl therapy. *Am. J. Vet. Res.,* 50, 1334—1339.

8. Geiser, D. R., Andrews, F., Sommerdahl, C., White, S., Maykuth, P., Younger, M. S., 1994: Electrolyte and acid-base changes in combinated training horses after the cross-country event. *Equine Practice,* 16 (7), 20—23.

9. Harkins, J. D., Kamerling, S. G., 1992: Effect of induced alkalosis on performance in Thoroughbreds during a 1,600-m race. *Equine Vet. J.,* 24, 94—98.

10. Hermansen, L., Orheim, A., Sejersted, O. M., 1984: Metabolic acidosis and changes in water and electrolyte balance in relation to fatigue during maximal exercise of short duration. *Int. J. Sport Med.,* 5, 110—115.

11. Kelso, T. B., Hodgson, D. R., Witt, E. H., Bayly, W. M., Grant, B. D., Gollnick, P. D., 1987: In **Gillespie, J. R. and Robinson, N. E. (ed.), 1987:** *Equine Exercise Physiology,* Vol. 2. ICEEP Publications, Davis, California, 438—447.

12. Kowalski, J., Roberts, A., Williams, J., Hintz, H. F., Daniluk, P., Schryver, H. F., 1991: Effect of interval between doses on response of the pony to sodium bicarbonate. *Cornell Vet.,* 81, 59—66.

13. Lawrence, L., Kline, K., Miller-Graber, P., Siegel, A., Kurcz, E., Fisher, M., Bump, K., 1990: Effect of sodium bicarbonate on racing Standardbreds. *J. Anim. Sci.,* 68, 673—677.

14. Lawrence, L. M., Miller, P. A., Bechtel, P. J., Kane, R. A., Krucz, E. V., Smith, J. S., 1987: In **Gillespie, J. R. and Robinson, N. E. (ed.), 1987:** *Equine Exercise Physiology,* Vol. 2. ICEEP Publications, Davis, California, 448—455.

15. Mastri, M., Freestone, J. F., Wolfsheimer, K. J., Shoemaker, K., 1990: Alternations in plasma volume, plasma constituents, renin activity and aldosterone induced by maximal exercise in the horse. *Equine Vet. J. Suppl.,* 9, 72—77.

16. Mckeever, K. H., Hinchcliff, K. W., Reed, S. M., Robertson, J. T, 1993: Role of decreased plasma volume in haematocrit alterations during incremental treadmill exercise in horses. *Am. J. Physiol,* R404—R408.

17. Piccione, G., Fazio, F., Caola, G., 1999: Cronoperformance nel cavallo saltatore: andamento circatrigintano di alcuni parametri emogasanalitici. *Atti So. Fi. Vet.,* 44—48.

18. Relman, A. S., 1986: "Blood Gases": arterial or venous? *New Engl. J. Med.,* 315—3, 188.

19. Rose, B. D., 1995: In **Mcgraw-Hill (ed.), 1995:** *Fisiologia clinica dell'equilibrio acido-base e dei disordini elettrolitici,* 233 pp.

20. Rose, J. R., Allen, J. R., Hodgson, D. R., Stewart, J. H., 1983: Responses to submaximal treadmill exercise and training in the horse: changes in haematology, arterial blood gas and acid base measurements, plasma biochemical values and heart rate. *Vet. Rec.,* 113, 612—618.

21. Sjogard, G., Adams, R. P., Saltin, B., 1985: Water and ion shifts in skeletal muscle of humans with intense dynamic knee extension. *Am. J. Physiol.,* R190—R196.

22. Taylor, L. E., Ferrante, P. L., Wilson, J. A., Kronfeld, D. S., 1995: Arterial and mixed venous acid-base status and strong ion difference during repeated sprints. *Equine Vet. J. Suppl.,* 18, 326—330.

23. Weil, M. H., Rackow, E. C., Trevino, R., Grundler, W., Falk, J. L., Griffel, M. I., 1986: Difference in acid-base state between venous and arterial blood during cardiopulmonary resuscitation. *New Engl. J. Med.,* 315—3, 153.

24. Zucca, E., Ferro, E., Ferrucci, F., 1999: Considerazioni sull'impiego dell'esame emogasanalitico nella clinica del cavallo. *Rivista Ufficiale SIDI,* 4 (2), 49—54.

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EXPERIENCE WITH FRONTOPARIETAL CRANIOTOMY IN A DOG — AN EXPERIMENTAL MODEL AND POSSIBLE CLINICAL IMPLICATIONS

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ABSTRACT

Craniotomy in a dog is a rather complicated procedure. Experience with this surgical approach gained during experimental procedures aimed at studying focal brain *ischemia* after permanent occlusion of one pericallosal artery performed on seven adult mongrel dogs of both sexes weighing 10—24 kg encouraged the authors to recommend their technique of *anaesthesia*, the preparation of the operation field, surgical technique, and postoperative treatment. Since all animals of the studied group survived the procedure without complications, the proposed measures could be exploited both in experimental as well as therapeutic operations on the skull, *dura mater*, brain and intracranial vessels in these animals.

Key words: craniotomy; dog; pericallosal artery occlusion

INTRODUCTION

Focal brain *ischemia* is the most frequent type of cerebrovascular event in humans (1, 4, 6, 12). It is caused by *thrombosis*, severe *stenosis*, coiling or kinking of arteries supplying the brain with blood, and results in the permanent disability of a patient or his/her death (3, 5, 6). The incidence of this clinical entity is slowly, but permanently growing with a tendency to appear in younger and younger persons. That is why focal brain *ischemia* due to the artherosclerotic vascular changes becomes a significant medical as well as social problem (1, 3, 4). The issue of spinal cord *ischemia* has been studied at the Neurobiological Institute of the Slovak Academy of Sciences in Košice for more than ten years (6, 8). This long-lasting interest has logically continued to the next step — the study of an ischemic stroke and further, the possibilities of influencing it therapeutically. The first problem to overcome was to find an appropriate experimental animal where the occlusion of an artery supplying a sufficiently large area of brain, could be performed to make the lesion manifest, but without the development of life-threatening damage (5, 12).

Having considered all circumstances, we decided to perform experiments on dogs, and it was the occlusion of a pericallosal artery that seemed to be the most suitable for our purposes. During experimental operations, the craniotomy in a dog turned out to be a rather complicated procedure, while the obliteration of one pericallosal artery involved, in the event, the use of microsurgical technique. Our approach could very probably be exploited also in other situations in the clinical practice of veterinary surgeons. This encouraged us to briefly comment on it.

ANIMAL MODEL AND OPERATIVE PROCEDURE

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

The occlusion of one of the pericallosal arteries at *genu corporis callosi* was performed through frontoparietal craniotomy in seven adult mongrel dogs of both sexes weighing 10—24 kg. The animals were anaesthetized with pentobarbital (*Pentobarbitalum natricum* — "Pentobarbital" SPOFA) administered intravenously in a 30 $mg.kg^{-1}$ dose, then intubated with endotracheal cannula (PORTEX, diameter 8—12 mm), and placed on a volume-cycled ventilator ("Anemat N8", CHIRANA) using a mixture of medical oxygen with 1—2 % narkotan (*Halothanum thymolum stabilisatum* — "Narcotan", LÉČIVA).

Continuous direct monitoring of their arterial blood pressure

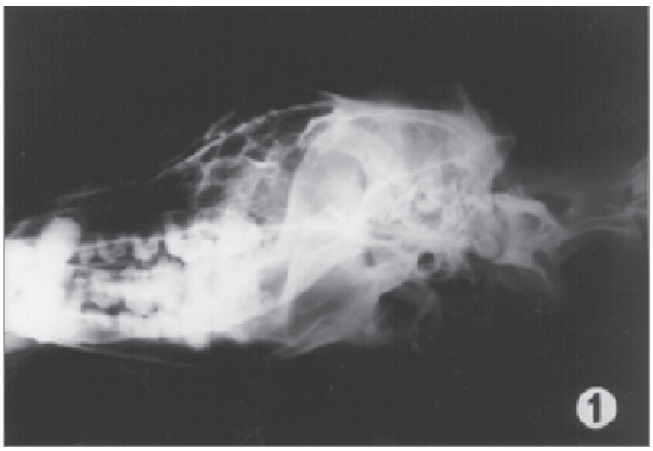

Fig. 1. Plain X-ray of a skull of the dog in lateral projection. Frontal sinuses reach almost to the top of the skull

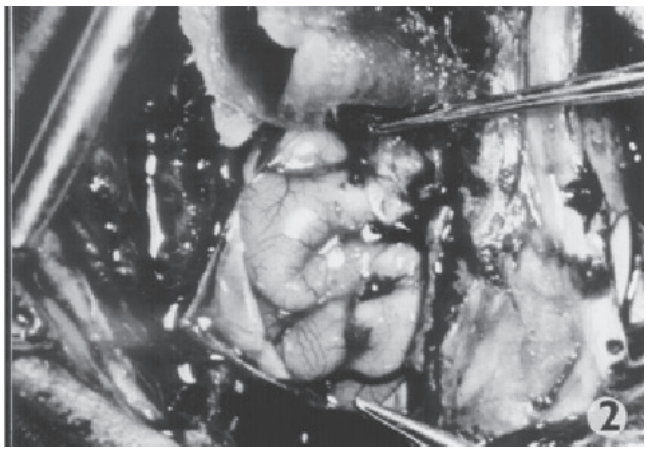

Fig. 2. Perioperative photograph of one dog from the experimental group. After craniotomy and durotomy, the brain partially covered by a piece of moist cottonoid is visible. Two fine forceps show extent of brain retraction during clip application on the right pericallosal artery

(monitor LMP 150, TESLA) and EKG (monitor LKM 220, CHIRANA) was carried out. The operating field was shaved, disinfected three times in succession by spraying with "SEPTONEX" (*Carbethopendecinii bromatum* spray, GALENA), and insulated from the surrounding area with sterile drapes. The operation itself started with a longitudinal midline incision from *protuberantia occipitalis externa* to the area of the *glabella*.

Using a scalpel, raspatory and bipolar electrocoagulation, the muscles (*musculus frontoscutullaris*, *musculus interscu- tullaris*, *musculus temporalis*, and *musculus corrugator supercili)* were loosened from the right side of the skull, shifted laterally and kept in this position by a self-retaining retractor. The parietal bone was trepanned by a tooth-drill and craniotomy extended to the desired size by removing parts of frontal and parietal bones by Luer rongeurs.

In a dog, frontal sinuses extend almost to the top of a head (Fig. 1). With the craniotomy performed with the aim of reaching a pericallosal artery at *genu corporis callosi* it was not possible to avoid their penetration. To diminish the danger of possible infectious complications, the opening in a frontal sinus was overlapped by a periosteal (pericranial) flap on a stalk. Potential bleeding from *diploe* was stopped by Horsley wax. The *dura mater* was lifted by a dural hooklet, incised by a pointed scalpel, and opened in a T-form with a base parallel to the superior *sagittal sinus* by special dural scissors. This way any possible damage to the neural tissue and its vessels was prevented. The brain was covered with cottonoids soaked in saline, bleeding was stopped by bipolar electrocoagulation, cerebrospinal fluid was removed by a water-operated suctior (Fig. 2).

After opening the *dura mater*, the operating surgeon inserted loops (ZEISS, magnification power 2, 3) for the better identification of important tiny structures. The right brain hemisphere was pushed away from the *falx cerebri* by a spatula and the right pericallosal artery was obliterated by a Heifetz anuerysmal clip. The operating field was rinsed with lukewarm saline, cottonoids renewed and the dural flap fixed back in place by a number of nylon stitches. The site of the craniotomy was covered by muscles, fixed to the midline by suturing their fascia, then subcutaneous tissue was sutured by catgut, and skin by nylon. During the operation, 1.500 000 u. i. of penicilline (*Benzylpenicillinum procainum*, BIOTIKA) and 1.5 g of streptomycine (*Streptomycin sulphate*, MEDEXPORT, Moscow) was administered intramuscularly to each of the experimental animals.

After completing the surgical procedure, the dog was placed into a separate, disinfected compartment, covered with warm blankets and monitored to the point of full awakening. The animals were administered drinking water *ad libitum* and offered a full diet. On the tenth postoperative day, one of the dogs was put under with a general anaesthesia, then perfused with 3.000 ml of saline and fixed with the same amount of 10 % neutral formaldehyde. The other six animals were left to survive, while healing of their wounds, neurologic state, and development of compensatory mechanisms were monitored.

RESULTS

All dogs survived the experimental operation without any complication. After awakening from general anaesthesia they refused to chew, but they drank water and from the next day milk as well. They started to accept solid food individually between the fifth and seventh postoperation day. The surgical wounds healed *per primam intentionem* in all animals of the studied group, so that on the tenth day after surgery the skin stitches could be removed. The clinical state of dogs was good, they were able to walk despite a light monoparesis of the left hind limb, that became obvious in a case of walking up on a sloping surface.

At the autopsy of the sacrificed animal, no significant pathological changes were discovered, but a residual swelling and discoloration in the medial part of the right brain hemisphere. The surgical wound healed without

inflammation, the *pericranium* overlapping frontal sinus was fixed by granulation tissue, the *dura mater* became water proof again.

Six of the experimental animals are still alive. As shown by the last follow-up examination carried out eight months after the craniotomy and clipping of the right pericallosal artery, they have been accepting typical food, their wounds have healed completely, and new hair has grown on their heads. Bone defects in the right frontoparietal region have been overlapped by muscles that provided the brain with a sufficient protection against accidental damage. A light monoparesis of the left hind limb has persisted in all the dogs from the group.

DISCUSSION

At present the therapeutic craniotomy in a dog is indicated mainly in a case of head injury or an intracranial tumour, which is closely associated with the improvement of diagnostic modalities and the introduction of appropriately modified neurosurgical procedures into veterinary medicine (10, 11). In the development of our experimental model we proceeded from the same principles, and in its implementation we employed the same technique and instruments as are used in human neurosurgery (11).

In a man, the *posterior cranial fossa* is overlapped by cervical muscles, a squamous part of the occipital bone is thin, and many of its irregularities considerably impede the insertion of a Gigli saw to perform the replaceable bone flap. That is why neurosurgeons when approaching the cerebellum, brain stem, and cranial nerves V—XII, remove pieces of occipital bone by rongeurs to the desired extent in order to gain access to the brain and tissue. The bone defect is covered merely by suturing up the cervical muscles over it in anatomical layers (11).

Only in the last year has experimental work appeared in non-human primates trying to enhance bone production in the area of craniotomy by the application of a bone morphogenic protein to the defect (9). Craniotomies in dogs of the experimental group were performed the same way. As shown by follow-up examinations the wounds healed almost perfectly and the scalp with muscles created sufficient protection layer over the defect in the skull. Apprehensions aboutf an almost inevitable penetration into paranasal sinuses, that in a dog can extend as far as the top of its head, and of a subsequent contamination of intracranial tissues by microorganisms from outside, have turned out to be unjustified (7).

The higher infection resistance of a dog, perioperative administration of antibiotics and overlapping the sinuses by a layer of healthy *pericranium* with preserved blood supply, prevented the development of suppurative complications in all animals of the monitored group. Similarly, the experimental model of a pericallosal artery occlusion through interhemispheric fissure, has turned out to be very suitable. However, the employment of proper illumination, magnification by surgical loops, fine instruments, and a microsurgical operative technique is advisable (2).

Having treated the vessels by bipolar electrocoagulation, protected the brain tissue with cottonoids soaked in lukewarm saline and drained off cerebrospinal fluid with a soft aspirator, there was no problem to draw the right brain hemisphere away from *falx cerebri*, and to obliterate the right pericallosal artery with an aneurysmal clip in the conventional way practised in neurosurgery (11, 12). The ischemic lesion was large enough to clinically manifest itself in the paresis of a contralateral hind limb and was reliably reproducible (neurologic deficit developed in all 7 dogs of the monitored group), but at the same time, it did not endanger the lives of experimental animals (5, 12).

CONCLUSION

In the authors' opinion, the above described technique can also be applied during operations on the intracranial cavity of a dog, when performed for different reasons, i. e. mainly in the treatment of injuries and tumours of convexity or medial surface of the brain and the anterior portion of *corpus callosum*.

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REFERENCES

1. Bartko, D., 1980: *Focal Brain Ischemia* (In Slovak). Veda, Bratislava, 676 pp.

2. El-Noamany, H. A., Nakagawa, F., Hongo, K., Kobayashi, S., 2001: Low anterior interhemispheric approach for anterior communicating artery aneurysms. In **McCulloch, G. A. J., Reilly, P. L. (eds.):** *Proceedings 12th World Congress of Neurosurgery,* Openbook Publ., Adelaide, 640—643.

3. Gdovinová, Z., Veselá, D., Eiben, E., 1999: Current state of diagnosis of brain vascular events and necessary measures of its improvement (In Slovak). *Folia Medica Cassoviensia,* 42, 156—159.

4. Gorelick, P. B., Alter, M. (eds.), 2002: *The Prevention of Stroke.* Parthenon Publ. Group, Boca Raton, London, New York, Washington, 266 pp.

5. Hacke, W., Schwab, S., Horn, M., Spranger, M., DeGeorgia, M., von Kummer, R., 1996: Malignant middle cerebral artery infarction: clinical course and prognostic signs. *Arch. Neurol.,* 53, 309—315.

6. Maršala, M., Danielisová, V., Chavko, M., Horňáková, H., Maršala, J., 1989: Improvement of energy state and basic modifications of neuropathologic damage in rabbits as a result of graded postischemic reoxygenation. *Exp. Neurol.,* 105, 93—103.

7. Miller, M. E., Christensen, G. C., Evans, H. E., 1968: *Anatomy of the Dog.* W. B. Saunders Co., Philadelphia, London, 941 pp.

8. Radoňák, J., Maršala, M., Maršala, J., 1999: Graded postischemic reoxygention as a preventive measure in spinal cord ischemia in experiments (In Slovak). *Rozhl. Chir.,* 78, 105—108.

9. Sheehan, J. P., Sheehan, J. M., Sheehan, H., Quigg, M., Helm, G. A., 2003: The safety and utility of recombinant human bone morphogenic protein-2 for cranial procedures in a nonhuman primate model. *J. Neurosurg.,* 98, 125—130.

10. Ševčík, A., Ledecký, V., Legáth, J., 1996: Incidence of complications during myelography in dogs (In Slovak). *Vet. Med.-Czech,* 41, 183—187.

11. Šteňo, J., 1995: Neurosurgical operative technique. In **Černý, J. (ed.):** *Special Surgery,* Volume 4. *Surgery of Neck and Head* (In Slovak). Osveta, Martin, 139—158.

12. Toda, S., Ikeda, Y., Teramoto, A., Hirokawa, K., Uekusa, K., 2002: Highly reproducible rat model of reversible forebrain ischemia — modified four-vessel occlusion model and its metabolic feature. *Acta Neurochirurgica,* 144, 1297—1304.

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LITHIUM CHLORIDE AS AN EXTERNAL MARKER FOR URINE PRODUCTION IN VEAL CALVES

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ABSTRACT

Lithium chloride was studied as potential external marker for total urine production in veal calves. Calves were fed a lithium chloride solution (250 g LiCl.l^{-1}) at levels of 0, 5, 10, 15, 20 and 25 millilitres per meal. The intake of lithium did not alter milk consumption. In the second experiment with twelve calves, lithium chloride solution (250 g LiCl. l^{-1}) was added to a reconstituted milk replacer presented in a plastic bucket.

On a group-mean basis, ingested lithium was fully recovered in the urine, the recovery being 104 %. Furthermore, on a group-mean basis urine production calculated from the amount of lithium ingested and the concentration of lithium in spot urine samples was similar to the total volume of urine collected, but this was not so for individual calves. It is concluded that lithium chloride is a valid external urine marker for milk-fed calves studied on a group-mean level.

Key words: lithium chloride; marker; urine production; veal calves

INTRODUCTION

Lithium is an alkali metal that is rapidly absorbed and excreted in urine (4). The normal level of lithium in plasma is less than 0.02 mg.l^{-1}. Lithium may be used as an external maker for the intake of supplementary food at pasture (2). Lithium chloride has also been used as a reference substance for urine collection in humans (1) and goats (3). Lithium recovery in urine was about 95 and 99 % for humans and goats, respectively. There are no data as to lithium chloride as possible external marker in veal calves. Under practical conditions of veal calf production, it is not feasible to collect urine quantitatively. An alternative method would be the use of a suitable external marker so as to estimate the amount of total urine production. In goats, it was (3) found that lithium chloride is a promising marker to estimate total urine production. We tested whether lithium chloride can be used to assess the total urine production in veal calves.

MATERIALS AND METHODS

Experiment 1

Sixty male Dutch Friesian-Holstein calves, about twenty-one weeks old, were used to investigate the effect of lithium chloride on milk consumption. The calves were housed individually in a ventilated room with wooden stalls. The calves were fed twice a day, at 0530 and 1730 hours, with a reconstituted milk replacer (eighteen litres per day) that was presented in a plastic bucket. The amounts added were either 0, 5, 10, 15, 20 or 25 millilitres of lithium chloride solution (250 g LiCl.l^{-1}) per meal. The solution was added in an equal volume per meal to the bucket before feeding the calves. The experiment lasted seven days and milk consumption and refusals were recorded daily.

Experiment 2

Twelve male Dutch Friesian-Holstein calves, about twenty-four weeks old, were used. The calves were kept individually in wooden stalls. They were fed a milk replacer supplemented with lithium chloride at a level of ten millilitres (250 g LiCl.l^{-1}) per meal. The calves were fed twice a day, at 0630 and 1630

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hours, with the reconstituted milk replacer presented in plastic buckets and ten millilitres of the lithium chloride solution was added to each bucket.

Collection of samples

After one week of lithium chloride ingestion, urine was collected quantitatively for five days. Urine production per day per calf was pooled and 100 mililitres was stored at –20 °C. On the third collection day, one hour after the morning feed, spot samples of urine (about twenty milliliters per calf) were collected from the plastic vials that were attached to the calf's belly. Milk consumption was measured during the five days of urine collection.

Chemical analysis

The urine samples were analyzed for lithium by atomic absorption spectroscopy (Varian AA-475, Varian Techtron Pty. Ltd., Springvale, Australia).

RESULTS

Experiment 1

All calves fully consumed the reconstituted milk replacers, but the calves fed either 0.5 or 10 millilitres of LiCl solution drank more rapidly than the calves fed more. There were no milk refusals. All calves were apparently healthy and showed no signs of sickness.

Experiment 2

There was no illness during the entire experimental period. The calves readily consumed the reconstituted milk replacers and no refusals were seen. The mean recovery of lithium in urine was 104 % (Table 1). Table 2 shows the urinary volume as based on quantitative collection and that calculated using both lithium intake and the lithium concentration in the spot samples. The group means of the two values were not significantly different. Figure 1 shows the correlation between the actual and calculated daily urine production in the veal calves ($r = 0.52$, $n = 12$, $P = 0.086$).

CONCLUSION

Lithium ingested was completely recovered in the urine. In this study we demonstrated that lithium chloride is suitable for use as an external marker for urine collection in veal calves on a group-mean level. However, in individual calves the use of lithium chloride as marker and urine spot sampling does not provide accurate estimates of total urine production.

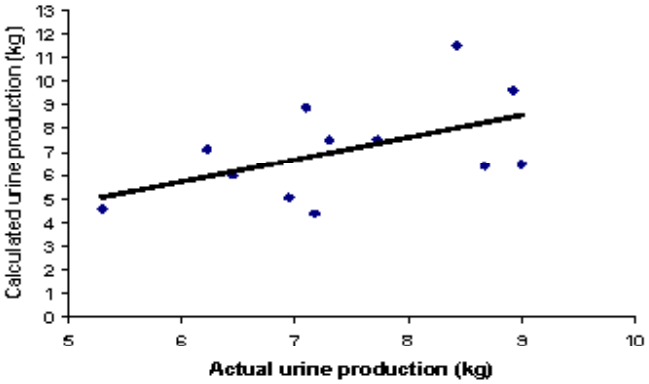

Fig. 1. Relationship between actual urine production and calculated urine production in individual calves fed milk replacers (data taken from Table 2)

Table 1. Lithium intake and urinary lithium excretion and recovery in twelve calves

Calf	Intake (mg/d)	Excretion (mg/d)	Recovery (% of intake)
1	809.5	857.6	105.9
2	809.5	872.3	107.8
3	809.5	792.3	97.9
4	809.5	926.3	114.4
5	809.5	802.6	99.1
6	809.5	883.7	109.2
7	809.5	848.1	104.8
8	809.5	787.1	97.2
9	809.5	783.2	96.8
10	809.5	876.2	108.2
11	809.5	865.4	106.9
12	809.5	824.4	101.8
Mean	809.5	843.3	104.2
SD		43.35	5.36

Table 2. Urinary volume as measured and as calculated for the third collection day

Calf	Actual volume, l	Calculated volume, l
1	8.43	11.51
2	9.00	6.48
3	5.30	4.55
4	8.68	6.39
5	7.10	8.90
6	6.23	7.12
7	8.93	9.63
8	6.45	6.01
9	7.18	4.37
10	7.73	7.51
11	6.95	5.05
12	7.30	7.52
Mean	7.44	7.09

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REFERENCES

1. Heijnen, M. L. A., Van Amelsvoort, J. M. M., Deurenberg, P., Beynen, A. C. 1996: Neither raw nor retrograded resistant starch lowers fasting serum cholesterol concentrations in healthy normolipidaemic subjects. *Am. J. Clin. Nutr.,* 64, 312—318.

2. Ralphs, M. H., Cheney, C. D. 1993: Influence of cattle age, lithium chloride dose level and food type in the retention of food aversions. *J. Anim. Sci.,* 71, 373—379.

3. Schonewille, J. Th., Beynen, A. C. 1999: Lithium chloride as a reference substance for urine collection in goats. *New Z. Vet. J.,* 47, 150—151.

4. Underwood, E. J., Suttle, N. F. 1999: *Mineral Nutrition of Livestock* (3rd edn.). CAB Publishing, CAB International, Wallingford, UK.

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USING PCR FOR DETECTION OF F18 FIMBRIAL ANTIGEN IN *E. coli* STRAINS ISOLATED FROM PIGLETS WITH DIARRHOEA IN SLOVAKIA

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ABSTRACT

One hundred and twenty seven *Escherichia coli* isolates obtained from piglets with diarrhoea across Slovakia were assessed for haemolytic activity and the presence of F18 fimbrial antigen. Haemolytic activity was detected in 85 % of all isolates. Thirty three (25 %) of the hundred and twenty seven *E. coli* isolates carried a gene for eighteen fimbriae. Of these thirty-three isolates, five strains were isolated from neo-natal diarrhoea and twenty-eight strains isolated from post-weaning diarrhoea. The PCR test used in this study was a sensitive and valuable method for determination of F18 fimbriae of *E. coli* strains.

Key words: *Escherichia coli*; F18; PCR

INTRODUCTION

Intestinal infections with enterotoxigenic *Escherichia coli* (ETEC) are an important cause of diarrhoea and mortality in humans in developing countries and in domestic animals. In pigs especially, ETEC infections immediately after birth *(neo-natal diarrhoea)* or later after weaning (*post-weaning diarrhoea*) are responsible for significant economic losses. ETEC is defined as containing the *E. coli* strains that elaborate at least one member of two defined groups of enterotoxins, heat-stable toxin and heat-labile toxin (8). Most ETEC strains possess adhesins, these are surface proteins called fimbriae and include: F4, F5, F6, F41 and F18. The fimbriae enable ETEC to colonize the small intestine of piglets by mediating their adhesion to the microvilli of epithelial cells (10).

Over the past few years considerable progress has been made towards the identification and characterization of a new type of F18 fimbrial *E. coli* isolated from piglets with diarrhoeal or oedemic disease (9, 12). However, little is known about the prevalence of F18 fimbriae in Slovakia. The aim of this study was to use a PCR to determine the prevalence of F18 fimbriae in *E. coli* strains isolated from *neo-natal diarrhoea* and *post-weaning diarrhoea* in Slovakia.

MATERIALS AND METHODS

Bacterial strains: One hundred and twenty seven *E. coli* strains isolated from piglets with diarrhoea were examined in this study. These *E. coli* strains were isolated from the intestinal content of carcases of piglets and from rectal swabs submitted to Department of Bacteriology, State Veterinary Institute, Nitra, Slovakia and to Institute of Microbiology and Immunology, Department of Food Hygiene and Food Technology, University of Veterinary Medicine, Košice, Slovakia during the years 2001 and 2002. The samples were cultured directly on MacConkey agar (Oxoid, Basingstoke, England) and were

identified as *E. coli using standard biochemical procedures. After isolation, the bacteria were stored frozen at –70°C after the addition of 20% glycerol. The strains represent fifty-four strains from piglets one to seven days old (neo-natal diarrhoea)* and seventy-three strains from piglets twenty-eight to forty-two days old *(post-weaning diarrhoea).*

Reference strains: A panel of reference *E. coli* strains was used as controls for PCR, and non-enterotoxigenic *E. coli* strains were used as negative control. *E. coli* positive for F18 was kindly supplied by Dr. Jacek Osek (12), National Veterinary Research Institute, Pulawy, Poland.

Haemolysis determination: The *E. coli* strains were cultured on sheep blood agar plates (37°C, 18 h) and the haemolysis zone was determined visually.

Isolation of DNA: Four or five colonies of tested culture were suspended in 100 ml of de-ionized water (MilliQ UF, Milipore) and boiled for ten minutes. Cell debris was removed by a short cycle of centrifugation (12.000 *g* for one minute) and 5 ml of the supernatant were used as the DNA of the sample (4).

Polymerase chain reaction: The primers were selected from sequences described by Imberrechts *et al.,* 1994 (6) and supply by Invitrogen (USA).

Primers:

FedA 1 5'-GTG AAA AGA CTA GTG TTT ATT TC-3'

FedA 2 5'-CTT GTA AGT AAC CGC GTA AGC-3'

Amplification of DNA was performed in a total volume of 50 µl. The reaction mixture contained: AmpliTaq DNA polymerase (Perkin Elmer) in the amount 0.5 U, 10-times concentrated PCR buffer (Perkin Elmer), dNTPs (final concentration 200 µM of each dNTP, dCTP, dGTP, and dTTP, respectively), magnesium chloride (final concentration 3 mmol), primers in final concentration 30 pmol, 5 µl of the DNA suspension and we added of de-ionized water to the total volume 50 µl (4, 5).

Table 1. Reaction conditions for F18 PCR reaction

Temperature	Time (min)	Nº of cycles
94 °C	5	1 – preliminary
94 °C	5	
55 °C	1	30
72 °C	5	
72 °C	5	1 – final

The amplified product was visualized by standard electrophoresis of 10 µl of the final reaction mixture on a 1.5% agarose gel. Amplified DNA fragments of specific sizes were located by ultraviolet fluorescence after staining with ethidium bromide. Their lengths were verified by 100 bp Ladder run simultaneously (Invitrogen). Control DNA from reference strains were included in each reaction.

Agarose gel electrophoresis: According the authors: 4, 5, 13.

RESULTS

Haemolysis determination. The α-haemolytic properties of *E. coli* strains tested were determined on blood agar plates. It was found that among the one hundred and twenty-seven isolates tested one hundred and eight strains (85%) were haemolytic. Among the thirty-three isolates known to carry genes for F18, twenty-nine strains (88%) were haemolytic and four strains were non-haemolytic.

Table 2. Haemolytic activity among 33 *E. coli* stains carry gene for F18 fimbriae

O-group (fimbrial antigen)	No. of strains	Haemolytic activity +	–
O147 (F18)	9	8	1
O141 (F18)	7	7	0
O138 (F18)	6	5	1
O139 (F18)	4	4	0
O163 (F18)	1	1	0
O157 (F18)	1	1	0
NT* (F18)	5	3	2
Total	33	29 (88 %)	4 (12 %)

*NT — Not typable

PCR analyses using PCR and primer pair FedA 1 and FedA 2, the *fedA* gene encoding the F18 fimbrial antigen was found in thirty-three out of one hundred and twenty-seven *E. coli* strains tested (25%). The amplified 510 bp PCR product (Fig. 1) was detected in strains isolated from both age groups of piglets with diarrhoea (*neo-natal diarrhoea* and *post-weaning diarrhoea*).

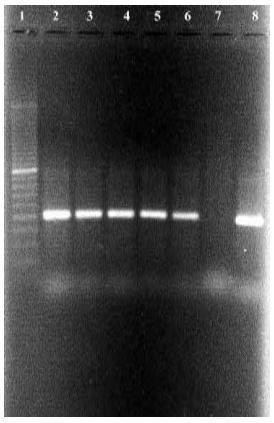

Fig. 1. Agarose gel electrophoresis of PCR-amplified *E. coli* DNA products
Legend (from the left): 1 — 100 bpLladder; 2—6 — PCR products F18 positive field isolates; 7 — Negative control; 8 — Positive control

Among fifty-four *E. coli* strains isolated from piglets with *neo-natal diarrhoea,* five strains (9.2%) were positive with F18. Of the seventy-three *E. coli* strains isolated from *post-weaning diarrhoea* twenty-eight strains (38.6%) were found to carry gene for F18 fimbrial antigen (Table 2).

Table 3. Prevalence of *fedA* among 127 *E. coli* strains isolated from piglets with diarrhoea

Piglets	No. of strains	No. of strains positive (%)	negative(%)
Neo-natal diarrhoea	54	5 (9.2 %)	49 (90.8 %)
Post-weaning diarrhoea	73	28 (38.4 %)	45 (61.6 %)
Total	**127**	**33 (25 %)**	**94 (75 %)**

DISCUSSION

This study was performed to assess the prevalence of F18 fimbrial antigen of *E. coli* strains isolated from piglets with diarrhoea in Slovakia. Information of this type is critical in order to understand the epidemiology of porcine strains better. Previous studies had been limited by lack of suitable and rapid assay, since F18 fimbrial antigen is rarely expressed under the usual cultural conditions.

In the present study the F18 fimbrial antigen and haemolytic activity of *E. coli* strains isolated from piglets with diarrhoea were investigated. It was found that most of the strains possessing the *fedA* gene were able to produce beta-haemolysin (29/33 strains). Wittig *et al.* (14) have also found that some *E. coli* strains isolated from pigs lost the haemolytic property under *in vitro* conditions. They suggest the presence of pathogenicity DNA islands on plasmids or on chromosomes, which can explain the correlation of the linkage gene clusters coding for haemolysin and fimbriae. However, we have found that *E. coli* strains can be *fedA* positive and haemolysin-negative or *fedA*-negative but able to produce beta-haemolysin on blood agar plates *in vitro*.

Using PCR analysis it was shown that thirty-three strains (25 %) had gene encoding a novel fimbrial antigen F18, which plays an important role in the colonization of the epithelial cells of the small intestine in weaned piglets by pathogenic *E. coli* strains (6, 7). Interestingly, in this study we found five strains (9.2 %) positive for F18 from fifty-four strains isolated from *neo-natal diarrhoea.* This finding is in agreement with Ojeniyi *et al.* (11), who found eight strains (4.2 %) out of 191 strains isolated from *neo-natal diarrhoea.* Also, Bischoff *et al.* (2) showed the presence of F18 fimbriae in forty-three strains (48 %) out of ninety *E. coli* strains isolated from *neo-natal diarrhoea* in the United States.

The fimbria F18 was first observed as an adhesive factor in connection with oedemic disease in pigs (1), but recently has been found present in strains connected with diarrhoea (3, 9, 12).These fimbriae are genetically different from the already known fimbriae, and are expressed *in vivo* only in the intestinal environment.

This work demonstrates that twenty-eight strains (38.3 %) out of seventy-three *E. coli* strains isolated from *post-weaning diarrhoea* carry the gene for F18 fimbriae. The percentage of F18-positive *E. coli* isolated from piglets with *post-weaning diarrhoea* described by other authors was similar to ours. Ojeniyi *et al.* (11) demonstrated that among F4-negative strains from weaned pigs with diarrhoea, a significant percentage (33.8 %) carried the F18 genes. Moreover, Wittig *et al.* (14) showed the presence of F18 fimbriae in 75 % of *E. coli* tested. More recently, Frydendhal (3) found 221 *E. coli* strains (39.3 %) out of 563 isolates positive for F18 fimbrial antigen in Denmark. On the other hand, Osek (12) and Kwon *et al.* (9) reported that the percentage of F18-positve *E. coli* strains isolated from *post-weaning diarrhoea was quite low in Poland (2.7 %) and Korea (1.83 %).*

CONCLUSION

This work indicates that the majority of all isolates are haemolytic. The PCR used in our study for detection of F18 fimbrial antigen was a valuable and sensitive method. The results obtained show preliminary epidemiological information about the presence of F18 in *E. coli* strains isolated from piglets with diarrhoea in Slovakia. Further analyses of a large number of strains are planned in the near future.

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REFERENCES

1. Bertschinger, H. U., Bachmann, M., Mettler, M., Posisschil, A., Schraner., E. M., Stamm, M., Sydler, T., Wild, P., 1990: Adhesive fimbriae produced *in vivo* by *Escherichia coli* O139 : K12 (B) : H1 associated with enterotoxaemia in pigs. *Vet. Microbiol.,* 25, 267—281.

2. Bischoff, M. K., White, D. G., McDermott, P. F., Zhao, S., Gaines, S., Maurers, J. J., Nisbet, D. J. 2002: Characterization of Chloramphenicol resistance in beta-hemolytic *Escherichia* associated with diarrhea in neonatal swine. *J. Clin. Microbiol.* 40, 389—384.

3. Frydendahl, K., 2002: Prevalence of serogroup and virulence genes in *Escherichia coli* associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches. *Vet. Microbiol.,* 85, 169—182.

4. Holečková, B., Holoda, E., Fotta, M., Kalináčová, V., Dianovský, J., Šiviková, K., Piešová, E., 2002: Enterotoxin production by *Staphylococcus aureus* in relation to food hygiene and ecology. *Folia Veterinaria,* 46, 2 (Suppl.), S32—S33.

5. Holoda, E., Pistl., J., Pilipčinec, E., 2001: *General Microbiology and Genetic of Microorganisms* (In Slovak). Vienala, Košice, ISBN 80-88985-62-5, 162 pp.

6. Imberechts, H., Bertchinger, H. U., Stamm, M., Sydler, T., Pohl, P., De Greve, H., Hernalsteens, J. P., Van Montagu, M., Lintermans, P., 1994: Prevalence of F107 fimbriae on *E. coli* isolated from pigs with oedema disease or postweaning diarrhea. *Vet. Microbiol.,* 40, 219—230.

7. Imberechts, H., Van Pelt, N., De Greve H., Lintermans, P., 1994: Sequences related to the major subunit gene fedA of F107 fimbriae in porcine *Escherichia coli* that express adhesive fimbriae. *FEMS Microbiol Lett.,* 119, 309—314.

8. Johnson, J. R., Pollar, D. R., Liod, H., Tyler, S. D., Rozee, K. R., 1990: Differentiation of genes coding for *Escherichia coli* verotoxin-2 and the verotoxin associated with porcine oedema disease (VTEC) by polymerase chain reaction. *J. Clin. Microbiol.,* 28, 2351—2353.

9. Kwon, D., Choi, C., Jung, T., Chung, H. K., Kim, J. P., Bae, S. S., Cho, W. S, Kim, J., Chae, C. 2002: Genotypic prevalence of the fimbrial adhesins (F4, F5, F6, F41 and F18) and toxins (LT, Sta, STb, and Stx2e) in *Escherichia coli* isolated from postweaning pigs with diarrhoea or oedema disease in Korea. *Vet. Rec.,* 12, 35—37.

10. Nagy, B., Fekete, P. Z., 1999: Enterotoxigenic *Escherichia coli* (ETEC) in farm animals. *Vet. Res.,* 30, 259—284.

11. Ojeniyi, B., Ahrens, P., Meyling, A., 1994: Detection of fimbrial and toxin genes in *E. coli* and their prevalence in piglets with diarrhoea. The application of colony hybridization assay, polymerase reaction and phenotypic assays. *J. Vet. Med. B,* 41, 49—59.

12. Osek, J., 1999: Prevalence of virulence factors of *Escherichia coli* strains isolated from diarrheic and healthy piglets after weaning. *Vet. Microbiol.,* 68, 209—217.

13. Toropila, M., Ahlers, I., Ahlersová, E., Ondrašovič, M., Beňová, K., Danko, J., Švický, E., Vargová, M., 2000: Changes in the activities of adaptive enzymes in laboratory rats during continuous gamma irradiation. *Folia Vet.,* 44, 17—20.

14. Wittig, W., Prager R., Stamm, M., Streckel, W., Tschape, H., 1994: Expression and plasmid transfer of genes coding for the fimbrial antigen F107 in porcine *Escherichia coli* strains. *Zentralbl. Bakt.,* 281, 130—139.

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CHRONICLE

Prof. MVDr. Ján Lazar (1928—1993)

This year we will remember that seventy-five years have elapsed from the birth and ten years from the death of Prof. MVDr. Ján Lazar, a long-standing dedicated and revered academic functionary, an important personality in Czechoslovak veterinary medicine, the head of the Department of Nutrition, Dietics and Foodstuffs of the College of Veterinary Medicine in Košice.

He was born on the 23rd July, 1928, in the village of Parchovany in the district of Trebišov (Slovakia). He graduated from the state secondary school in Michalovce where he also passed his school-leaving examinations. After his examinations, he enrolled at the College of Veterinary Medicine in Brno where he studied from the year 1948 to 1953. He obtained his veterinary diploma on the 26th July, 1953, and graduated on the 12th December, 1953, after a successful defence of his dissertation thesis on the theme *"Contribution to the Explanation of the Dietary Effect of Rapeseed Cake"*. Already during his studies he had been employed as a trainee assistant at the Department of Nutrition and Dietics. After graduation from the college he became assistant at this department.

His professional interests affected his life and further work. An important milestone in his life was in September 1958, when, at the request of the dean of Veterinary Faculty of the Agricultural College (AC) in Košice, he moved to Košice and was appointed the head of the Department of Nutrition and Dietics and held this position till 1989, that is more than 30 years.

After successful defence of his habilitation thesis *"The Study of Carotenes in the Diet of Dairy Cows"* he was appointed assistant professor on the 1st April, 1963, and then deputy professor on the 1st August, 1965. In 1980 the president of ČSSR appointed him a university professor.

In the period from 1963 till 1969 he held the post of dean of the Veterinary Faculty of AC and from 1969 until 1972 of the rector of the College of Veterinary Medicine in Košice. He held these academic posts in a very complex political period which was also rather difficult for the College. As a leading functionary, he was forced to deal with very unpleasant and essential issues. He always tried to solve the problems with prudence. Despite the complexity of this period, the years mentioned were very successful in the pedagogic-educational, scientific-research and construction investment sectors. From 1980 to 1985 he was a vice-rector for scientific research and contacts with foreign countries.

19th December, 1968, was an important milestone for the College as its independence was restored on the basis of a specific government Act. During its 29th meeting the National Assembly approved the governmental proposal of Act No. 169 on the re-establishment of Colleges of Veterinary Medicine in Brno and Košice. At the beginning of the academic year 1969/1970, the Veterinary Faculty of AC in Nitra became the College of Veterinary Medicine in Košice. According to § 3, veterinary colleges are schools of a university type. Deans of both faculties concerned, Prof. MVDr. Ján Lazar and Prof. MVDr. Evžen Novotný, made a considerable contribution to the passage of this Act.

As a dean and later also rector of the new College, Prof. Lazar undertook additional intensive development of the school so it could fulfil the increasing demands on its professional expertise and scientific level. From 1966 to 1967 a general reconstruction of the entire pavilion XII took place, intended for the Department of Nutrition and Dietics. In 1971 a student dormitory was completed as a very spacious new building. The biggest construction ever realized on school premises, associated with his name, was the clinical pavilion serving three clinics that was completed in 1976. The fourth clinic was supposed to be placed in pavilion VII but this plan was not implemented.

In 1970 a specific school-establishment, the Centre for Gamekeeping and Diseases of Game, was founded and built in Rozhanovce which contributed to the development of the respective scientific subject and was affiliated to the Department of Nutrition and Dietics. His person was associated also with establishing the College Agricultural Plant in Zemplínska Teplica. In 1972 the development of an Experimental Research Centre was initiated in Zemplínska Teplica and its operation started on the 1st June, 1984.

The life of Prof. Lazar was filled up with intensive research, educational, organisational and consultation activities. He was indefatigable and energetic in all activities concerning development of the College but also outside it. In his capacity as the rector he contributed to the extension of collaboration with other schools abroad. His diverse involvement and organisational activities were related to his work in various scientific and specialised bodies. He held several specialised functions: member of Federal Commission Conferring State Awards, member of the Division of Animal Production of ČSAP, member of Veterinary Division of SPA, chairman of Commission for Nutrition of the Professional Board of State Veterinary Administration of SR. He was a member of Scientific Collegium for Special Biology in the Slovak and Czechoslovak Academy of Sciences. He was also a chairman of Commission for Defence of PhD. Thesis in the field of nutrition and diet of farm animals and of other commissions for thesis defence in Košice and Brno.

The research activities of Prof. Lazar were also extensive. Under his leadership the members of his department focused on the study of the influence of vitamin and mineral nutrition on production and reproduction of farm animals and the quality of animal products. In addition to this, his investigations included the influence of some ecological factors and the relationship between increased use of chemicals in plant production and the nutrient and dietary value of fodder and feed that was reflected in the health status of animals (1971—1975). Beginning in 1986, attention was paid to the protein nutrition of sows in relation to increasing their productivity.

Under his leadership, fifteen PhD. thesis (ten by veterinarians and five by agricultural engineers) and one doctor's dissertation were prepared at the Department and defended successfully.

After the establishment of the Institute of Experimental Veterinary Medicine in Košice in 1970 he became the head of its Department of Veterinary Dietics. He directed the research of this department on the influence of differentiated chemicalisation on the yield of some feeds and their dietary value, on lipophilic vitamins in animal nutrition and the use of waste products. Owing to his initiative, utilization of waste from the production of penta-erythritol was resolved in the form of syrup Penta that was supplied for fattening cattle (1985). Later on his team prepared a proposal for computer-controlled optimisation of rations intended for the new technology of the milk feeding of calves.

It should be stressed that from 1971 till 1990 Prof. Lazar was a coordinator first of the principal task of the state plan of basic research and later a chairman of the co-ordination board of key directions of the Target Programme of the State Plan of Basic Research controlled by the Czechoslovak Academy of Sciences. He acquitted oneself well in this demanding and responsible work.

From 1971 to 1975 the College of Veterinary Medicine in Košice, represented by Professor Lazar, was again entrusted with solving, co-ordinating and acting as guarantor of the key direction of the Target programme No. VI-6 *"Theoretical Basis of Health and Morbidity of Animals"* The co-ordinator of the programme was again Professor Lazar. The same situation occurred from 1976 to 1980.

From 1981 to 1985 he was involved in the Target programme of Key Orientation VI-5: *"Theoretical Basis of Agriculture — Health, Productivity and Diseases of Animals"*. From 1986 to 1990 the College of Veterinary Medicine in Košice participated in resolving, providing guarantee and co-ordinating the Target Programme of Key Orientation VII-4: *"Farm animals – Theoretical Basis of Increasing Productivity and Protecting the Health"*.

As a research worker in the field of veterinary vitaminology he was one of the team members and a co-ordinator of the research task within RVHP states on the theme *"Improvement of Vitamin Nutrition of Animals"* in the co-ordination centre in Dummersdorf near Rostock. The outcome of this collaboration consisted of a specification of standards concerning the requirements of farm animals for some vitamins and a proposal of unified methods for the determination of lipophilic vitamins.

The results of his research were published in various scientific and professional journals at home and abroad and in proceedings of symposia and conferences (about two hundred papers).

He was an author/co-author of the following books: Kábrt, J. and Lazar, J.: *"The Nutrition and Diet of Farm Animals"*. SZN Prague, 1963; Fried, K. *et al.: "Vademecum of a Veterinarian"*. 1st edition, Príroda, Bratislava, 1969 (Nutrition of domestic animals, pp. 21—67).

He prepared and published the following lecture notes and treatises: Lazar, J.: *"The Nutrition and Diet of Farm Animals"*. SVPL Bratislava, 1963; Lazar, J. and Rosival, I.: *"The Computer-controlled Formulation of Rations for Cattle"*. Príroda, Bratislava, 1982; Lazar, J. *et al.*: *"Introduction to the Utilisation of Computers in Animal Nutrition"* Príroda, Bratislava, 1984; Rosival, I., Lazar, J. and Beseda, I.: *"Computers in Diagnostics and the Prevention of Health Problems Caused by the Improper Nutrition of Cattle"*. ÚVIO, Bratislava, 1985.

He was a hard-working and sensitive person with a social sensitivity and a sense of justice. He hated selfishness, caginess, duplicity and cunning. He respected assiduous work and tried to evaluate it objectively and without bias. For this he was generally respected.

He was also an open and sociable person and enjoyed to be surrounded by people who understood him. However, he would flare up and raise his voice when he met with biased and superficial conduct or ungrounded invectives against his friends or himself. He took a long time to forget any wrongdoing.

His important working results were awarded with high honours, such as the state distinction *"Of Merit for Development"; "Gold Medal of Prof. Dr. P Adámi", "Gold Medal for Merits for Development of Biological Sciences of the Slovak Academy of Sciences", "Golden Plaque of the Czechoslovak Academy of Sciences", "Bronze and Gold Medals of ČSAP"* (1978 and 1988). Branch awards *"Exceptional Worker in Agriculture and Nutrition", "Merited Worker of the State Veterinary Administration of the SSR",* and others.

He retired early on the 31st October, 1991 for health reasons. From the 1st October, 1992, until the 30th June, 1993, while a pensioner, he held a part-time job in scientific research.

Due to serious illness he passed away on 24 August, 1993, at the age of 63.

Jantošovič, J., Bugarský, A., Kozák, M.
The Museum of Veterinary Medicine
– The UVM in Košice

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The 135th anniversary of Prof. Dr. J. Marek

Professor Doctor Jozef Marek is one of the most important representatives of world veterinary science. With his lifelong work and extensive scientific studies he tried his utmost to raise veterinary medicine to a high professional level. He contributed significantly to the development of internal veterinary medicine on a world-wide scale.

He was born on the 18th March, 1868, in Horná Streda nad Váhom (district Piešťany at that time) into a small-peasant family. He attended the higher classes of the King's Roman Catholic Secondary Grammar School in Prešov as a novice of a minor order and later he attended the Archbishopric Higher Secondary School in Trnava. There he completed the 8th year and passed the school-leaving examination with honours on the 12th June, 1888. The 8th form annual report No. 16 stated: *"speaks Slovak, Hungarian and German",* that is his native language was mentioned first.

On the 5th November, 1892, he obtained a veterinary diploma at the former Veterinary Academy in Budapest. Then, for a short time, he practised as a veterinarian in Nové Mesto nad Váhom. In 1894 he passed the physician's examination and became a medical officer. On the 8th July, 1895, after little less than three years of veterinary practice, he was appointed the head of the laboratory of the Country Animal Health Office in Budapest. In April 1897, upon the recommendation of the director of Academy, Professor Doctor F. Hutyra (a native of Spišské Podhradie — Slovakia) the Minister of Agriculture appointed him the post of clinical adjunct and at the same time granted him a one year study vacation abroad *"to be able to acquire, in addition to a special study of clinical scientific branches, also a doctoral qualification"*. To fulfil this goal he enrolled in the Philosophical Faculty of the University in Berne in Switzerland where he submitted his doctoral thesis on the theme *"On the Helvetian-Gaulish Horse and His Relationship to Pre-Historical and Recent Horses"* on the basis of which he was promoted with honours to Doctor of Philosophy on the 25th April, 1898.

After his promotion he visited a number of European veterinary schools and enrolled as a special student in the Medical Faculty in Vienna in order to study the diagnostics of internal diseases in its Internal Clinic. Upon returning from his study stay he took up the function of a clinical adjunct. On the 8th August, 1898, the branch minister appointed him the head of the Clinic of Internal Diseases and a lecturer. Shortly after that (January 30th, 1901) he was appointed Professor.

Professor Marek enriched veterinary medicine with many original and crucial observations in the field of clinical diagnostics, symptomatology and also prevention. He directed his research activities towards various, relatively diverse problems. In the beginning he focused on electrodiagnostics (1899) and the physical examination of lungs (1901—1903). He improved nasopharyngeal probing in horses (1905) and developed a method of examination of the upper airways and air sac in odd-toed ungulates by means of a laryngoscope (1911).

He was the first to study the histopathology of the nervous system in Aujeszky's disease and termed it an infectious bulbar paralysis of pigs (1902); he described in detail the clinical picture of this infectious disease. He investigated the essence of neurolymphomatosis of gallinaceous fowl (1907) which was later named after him as Marek's paralysis. He developed a reliable anti-fluke preparation Distol.

However, his long-term (1928—1944) research efforts focused on the complex of metabolic osteopathies. He described the results of his studies in dozens of papers and published them in a number of scientific and professional journals.

Professor Marek was an author of many excellent books that gained world-wide recognition. Together with Professor Hutyra he published a monograph on eastern rinderpest with special stress on its clinical symptomatology and pathological and differential diagnostics under the title: *"Die orientalische Rinderpest mit besonderer Berücksichtigung der klinischen und atomischen Merkmale und der Differentialdiagnose"* (publishing house G. Fischer, Jena, 1906).


SPEZIELLE
PATHOLOGIE UND THERAPIE
DER HAUSTIERE
VON
DR. FRANZ v. HUTYRA DR. JOSEF MAREK
DR. RUDOLF MANNINGER
ZWEITER BAND
ORGANKRANKHEITEN
BEARBEITET VON J. MAREK
VERLAG VON GUSTAV FISCHER IN JENA

He devoted more than fifteen years to the extensive and detailed experimental and comparative study of aetiology, biochemistry, pathogenesis, pathological anatomy and clinical symptomatology of rachitis and its relationship to related bone diseases. Together with Oscar Wellmann he published the results of his study in a two-volume monograph: *"Die Rachitis in ihren äthiologischen, biochemischen, pathogenetischen, pathologisch-anatomischen und klinischen Beziehungen"* (the pathological part by Marek was published in 1931 and the biochemical by Wellmann in 1932; G. Fischer, Jena). This monograph was published also in Hungarian under the title: *"Az angolkór oktani anyagforgalami, kórfejlödéstani, kórbonctani és klinikai vonatkozásaiban"* (Budapest, 1930—1932).

Marek's name became known round the world due to his excellent work — a textbook on the clinical diagnosis of skin and internal diseases of domestic animals. Due to its nature and importance it was included among the most important books in this scientific branch of veterinary medicine: *"Lehrbuch der klinischen Diagnostik der inneren Krankheiten der Haustiere"* (G. Fischer, Jena). The first edition was published in 1912, the second in 1922, the third in 1937 and the fourth in 1951 (in collaboration with J. Mócsy). The fifth and sixth editions, reworked by Mócsy, were published in 1956 and 1960, respectively. The book was published originally in Hungarian under the title *"Klinikai diagnosztika" (*Budapest): the first edition in 1902, second revised and supplemented edition in 1928 and the third and fourth editions were published by Mócsy (the fourth one was published in 1944).

Another book focused on internal medicine: *"Állatorvosi belgyógyászat"*. He published it in Budapest together with Hutyra, the first edition in three volumes in 1904 and the second in two volumes in 1923—1924. The favourable acceptance of this book by Hungarian veterinarians stimulated the authors to think about its publishing in one of the world languages. After some consideration they selected German. This resulted in the publication of a standard work on the special clinical pathology, organ therapy and infectious diseases of domestic animals *"Spezielle Pathologie und Therapie der Haustiere"* (G. Fischer, Jena) through which the authors achieved fame round the world. The first edition was published in 1905 and there have been ten additional ones in German since (the second in 1909, the third in 1910, the fourth in 1914, the fifth in 1920, the sixth in 1922, the seventh in 1938, the eighth in 1941, the ninth in 1945 and 1952, the tenth in 1954 and the last, the eleventh, in 1959). All editions were published in two volumes except the sixth which consisted of three volumes. The first to sixth editions were prepared by Hutyra and Marek; R. Manninger participated in the seventh and eleventh and Mócsy in the ninth to eleventh. The eleventh edition was translated into Russian and published in Moscow. Later on there were three editions in Italian, two in Russian, two Soviet, three Anglo-American, two English, two Spanish, one Turkish and one Serbian (Böhm, 1972). The monographs were translated also into Polish and Chinese. Part of them were translated also into Finnish.

Doctor Marek's successful scientific efforts gained him the highest honours: the Order of the Honourable Cross of Francis Joseph (1916), six honorary doctorates, honorary membership of the Hungarian Academy of Sciences (1942), the Kossuth State Award (1949), membership of the French Veterinary Academy; membership of the British Royal Veterinary Society and American Veterinary Society, honorary membership of four European veterinary societies.

After the death of Professor Schindelka (1913) the Veterinary College in Vienna offered Marek the vacant position of the head of Department of Internal Diseases; however he did not accept this honour neither did he accept the attractive offer of the Turkish government of 1923 to become the rector of

the Veterinary College in Istanbul that required reorganisation.

In 1919 Marek was supposed to become a professor at the newly established College of Veterinary Medicine in Brno. In the beginning, following the political chaos in Hungary, he seemed to be willing to move to Brno. However, after much hesitation and particularly owing to the advice of his teacher and then rector Professor Hutyra, who perceived it as ingratitude towards his alma mater, he decided to stay in Budapest.

He never forgot his Slovak birthplace, Horná Streda nad Váhom, where he returned regularly when he could to visit the graves of his parents and both his deceased wives. His feelings for his homeland and native language did not lessen even with a long-term absence. All the letters sent to his relatives were written in Slovak.

Everybody considered it an honour to see Professor Marek, to meet him and talk to him. Several veterinarians from Slovakia were in contact with him either through letters or, occasionally, in person. One of them was Professor Hrudka who met him in Budapest as the only one teaching at the College of Veterinary Medicine in Košice.

In 1935, upon his own request, Marek retired. He passed away on September 7, 1952, in Budapest and was buried there. His wish was to rest in the family crypt in Nové Mesto nad Váhom where both his wives were buried. This wish, however, did not come true.

On the occasion of the 2nd anniversary of his death (1954) a monument to his honour was unveiled at the premises of the College of Veterinary Medicine in Budapest. In 1994 a College dormitory was named after him and his bust was set in its lobby.

Since 1975 the University of Veterinary Medicine in Budapest has awarded the commemorative medal of Dr. J. Marek to recognise merit in the field of internal veterinary medicine. Of Slovak veterinarians this medal has been awarded to Academician O. J. Vrtiak (1975) and Prof. Dr. J. Rosocha (1977). On the 16th June, 1988, a commemorative plate to Jozef Marek was unveiled in his hometown. Ten years later, on the 27th March, 1998, on the occasion of the 735th anniversary of the first historical reference to Horná Streda and the 130th anniversary of the birth of Professor Marek day-long festivities were held to celebrate these anniversaries. On this occasion, in the presence of numerous guests, Prof. Dr. František Hrudka, DrSc., and the village magistrate, the academic sculptor, Jaroslav Hladký, unveiled a relief of Professor Marek.

The Commemorative plate and relief, located on the Cultural centre in Horná Streda will remind all visitors and his fellow countrymen that in 1868 one of the most important representatives of world-wide veterinary medicine was born in this village. In this way the village inhabitants and Slovak veterinarians have repaid, at least in part, their moral debt to their renowned countryman.

Jantošovič, J., Bugarský, A., Kozák, M.
The Museum of Veterinary Medicine
– The UVM Košice

Linz 2003

11th Congress on Alternatives to Animal Testing
19th - 21st September 2003
University of Linz Austria

8th Annual Meeting of MEGAT -
Middle European Society for Alternative Methods to Animal Testing

Subjects

Animal welfare and ethical aspects
Xeno-transplantation, embryonic vs. adult stem cells: new aspects relevant to the 3Rs

Standardization of cell cultures
main focus: Strategies to replace fetal bovine serum (FBS)

Toxico-genomics
The possibilities of testing "chronic toxicity" in vitro

RNA-Interference
A new method for testing transgenic cells without animal experiments

In vitro Testing of "chronic toxicity"

The 3Rs in ecotoxicology
Industrial and environmental aspects

In vitro alternatives to animal experiments and the EU white book on a new chemicals policy

Current positions of the 3Rs in development, production and testing of therapeutic antibodies

The 3Rs in academic education

Free communications

Organisers

Organiser

- zet - Zentrum für Ersatz- und Ergänzungsmethoden zu Tierversuchen (Centre for Alternative and Complementary Methods to Animal Testing), A-Linz

Co-organisers (in alphabetical order)

- biomed - Verein zur Förderung der biomedizinischen Forschung in OÖ, A-Linz
- Department for Austrian and International Commercial and Economic Law of the University of Salzburg, A-Salzburg
- Doerenkamp-Zbinden-Foundation, D-Erlangen
- ECVAM - European Centre for the Validation of Alternative Methods, I-Ispra
- FFVFF - Foundation for Animal-Free Research, CH-Zürich
- Institute for Animal Husbandry and Animal Welfare of the University of Veterinary Medicine Vienna, A-Vienna
- Institute for Biomedical Research of the University of Graz, A-Graz
- Institute for Medical Physics and Biophysics of the University of Graz, A-Graz
- Institute of Physiology and Balneology, University of Innsbruck Medical School, A-Insbruck
- MEGAT - Middle European Society for Alternative Methods to Animal Testing, A-Linz
- set - Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuch, D-Mainz
- Steinbeis-Technology-Transfer-Center for In-vitro Pharmacology and Toxicology, D-Konstanz
- Upper Austrian University of Applied Sciences, School for "Medical Technology", D-Konstanz
- ZEBET - Centre for Documentation and Evaluation of Alternatives to Animal Testing - at the BfR (Federal Institute for Risk Assessment), D-Berlin

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