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THE PRESENCE OF NERVE FIBRES IN THE GUT LYMPHOID TISSUE OF THE CAT

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ABSTRACT

The aim of the study was to follow the distribution of the nerve fibres in the gut lymphoid tissue in the cat. For this purpose an indirect immunohistochemical method and acetylated α-tubulin antibody was used. Two portions of the intestine were involved in this study: the ileum and colon. Nerve fibres for lymphoid nodules arise from the nerve plexus located in the submucous and mucose connective tissue. Most of the related nerve fibres were seen in the submucosal space and at the base of the lymphatic nodules in a relationship with blood vessels. From the submucous layer nerve fibres run in septa radially between the ileal internodular space of Payer's patches. Fine nerve fibres reach the lining gut epithelium at the sides and top of the dome. In the colon nerve fibres run around the single lymphatic nodules. Positive enteric nerve fibres are in both organs, located at the periphery of the lymphatic nodules. Only a few nerve fibres enter the peripheral zone of the lymphatic nodules and no positive nerve fibres were found in the central area of the lymphatic nodules of the ileum and colon.

Key words: cat; gut; lymphatic tissue; innervation; immunohistochemistry

INTRODUCTION

There is evidence that there is a functional interaction between the immune and nervous system (2, 11, 1). Neurotransmitters and neurohormones can act through receptors on cells of the immune system to modulate their activity and cytokines can interact with central neurons to modulate their activity. These interactions can exert a profound influence on functional activity in both systems and may play a major role in the pathogenesis and progression of disease (5). In the intestine, the enteric nervous system is held to be involved in the regulation of immune responses.

Garcia-Arraras and Viruet (7) have noted that anti-acetylated alfa-tubulin can be used as a marker for nerve fibres in the enteric nervous system. For this purpose an indirect immunohistochemical method and acetylated alfa-tubulin were used in our study. The aim of the present work was to demonstrate the nerve fibres in the wall of the rabbit ileum with special attention to the lymphoid tissue of the ileum and colon of the gut of the cat.

MATERIAL AND METHODS

The study was performed on six adult cats of both sexes. Samples of the ileum and caecum were removed and placed in 0.1 mol phosphate buffered 4 % formaline for twenty-four hours at room temperature, dehydrared and embedded in paraffin. Sections 5 μm thick, were stained with haematoxylin-eosin or processed following the avidin-biotin-peroxidase complex (ABC) method (10). Following deparaffinisation, the sections were hydrated, incubated for twenty minutes 0.3 % H_2O_2 in PBS to reduce endogenous activity and preincubated with 2 % goat serum to mask non-specific binding sites. Afterwards the sections were incubated overnight with the first monoclonal mouse anti-acetylated tubulin antibody, clone 6-11B-1 (Sigma), dilution 1:1000. Afterwards, the sections were washed twice in PBS and then incubated with goat anti-mouse biotinylated immunoglobulin at 1: 20 dilution. After one hour of incubation with a secondary antibody, the sections were incubated with ABC complex and developed with 0.05 % 3´.3´-diaminobenzidine (DAB) and 0.03 % v/v H_2O_2. Some sections were counter- stained with Mayer's haematoxylin. Negative controls were performed by omiting the primary antibody.

Fig. 1. Section through the circular muscular layer and the submucosa of the ileum. Bands of fine nerves are present inside the muscle layers and the submucosa. A few nerve fibres enter between two lymphatic nodules of Payer's patches (*arrow*) (× 150)

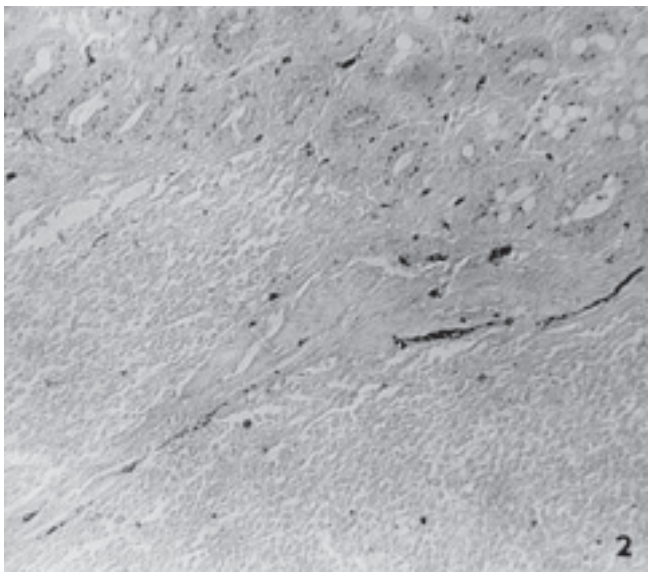

Fig. 2. Section through the submucosa and mucosa layer. A fine nerve fibres run between the lymphatic nodule of Payer's patches and reach the mucosa (× 170)

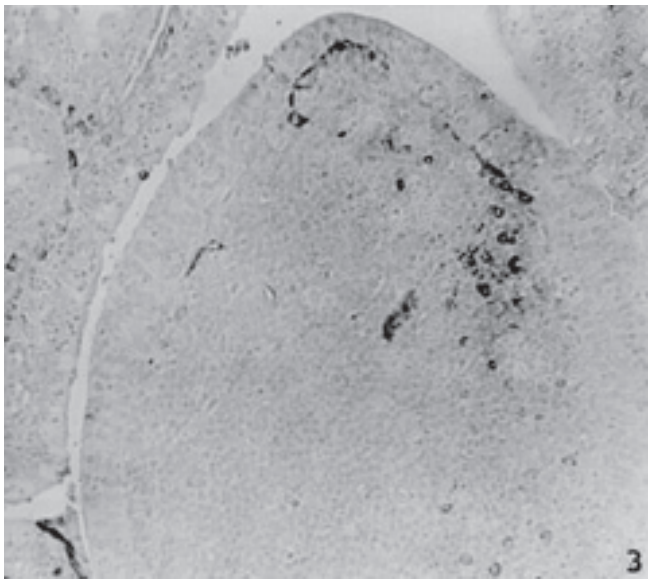

Fig. 3. Section through Payer's patches in the ileum. Fine nerve fibres are seen beneath the lining epithelium on the lateral side and at the top of the dome (× 170)

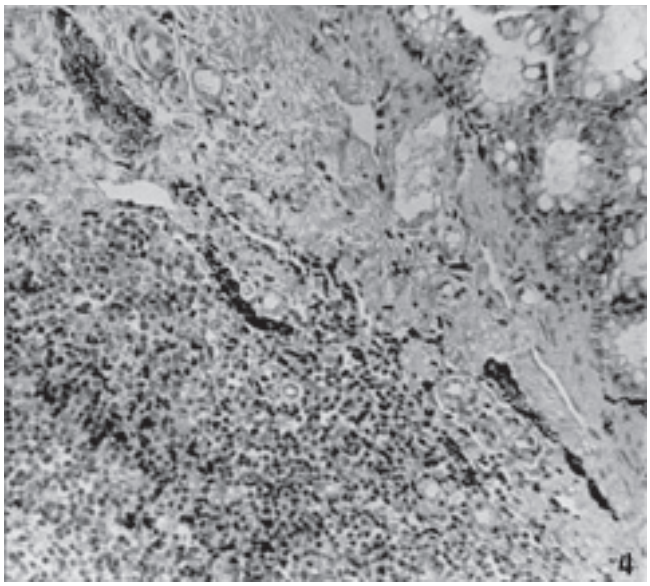

Fig. 4. Section of part of the submucosa and mucosa layer of the colon. Thin nerve bands are present on the periphery of the lymphatic nodule in close contact with small blood vessels (× 200)

RESULTS

Most of the nerve fibres related to lymphoid nodules in both portions of the gut were seen in the submucosal space and in mucosal *lamina propria* located next to the lymphatic nodules as ganglionic submucosal nerve plexuses and a ganglionic mucosal plexus. Due to the location of the lymphoid nodules of both portions of the gut in the space of the *tela submucosa* and *tunica mucosa* there is a close topographical relationship of the nodules with both plexuses. There were no concentration of the nerve fibres related to the lymphatic nodules. Only a few fine nerve fibres were seen to leave the submucosal plexus and to enter the space of the nodules of Payer's patches.

The fine nerves positive to acetylated tubulin innervating the lymphoid tissue of the gut arise from the ganglia of the submucosal plexus (Fig. 1). In Payer's patches the nerve fibres were seen to be located in the connective tissue of the submucosa and partially involved in connective tissue of the *lamina propria* of the mucosa which make a delicate capsule around them (Fig. 2). Nerve fibres that are detached from the submucosal plexus run

radially in the connective tissue of the interfollicular space of Payer's patches and pass around the nodules reaching the peripheric lateral side of the nodules. Only a few of these fibres enter the peripheric zone of the lymphatic nodules. In Payer's patches there are delicate enteric nerve fibres passing among the lymphatic nodules to reach the apical zone of the domes. Here the nerve fibres run under the associated epithelium and are seen on the lateral side or at top of the dome (Fig. 3).

In the colon small bands of fine nerve and single nerve fibres, running under the *lamina muscularis* of the mucosa layer, were seen. Some of these bands and fine nerve fibres enter the periphery of the nodule (Fig. 4). As in the single lymphatic nodule of the colon and in the nodules of Payer's patches we did not find nerve fibres inside the central area of the nodules. There were no differences in the distribution of the nerve fibres inside the lymphatic nodules among the Payer's patches of the ileum and the solitary lymphoid nodules of the colon.

DISCUSSION

The lymphoid tissue is organized differently in individual lymphoid organs and the organization of innervation also presents differences. Felten *et al.* (4) in a variety of mammal species have narrated that the sympathetic noradrenergic nerve fibres innervate both the vasculature and parenchymal fields of lymphocytes and associated cells in several lymphoid organs, including gut-associated lymphoid tissue. According to the authors this innervation is both regional and specific and generally is directed into zones of T-lymphocytes and plasma cells rather than into nodular regions or B-lymphocyte regions.

The organization of the lymphoid tissue effect on their innervation in the gut lymphoid tissue of the cat corresponds with the authors cited above. We were unable to find nerve fibres inside the B-lymphocyte area of the lymphatic nodule though many nerve fibres form a dense network under the follicle or some of them enter the periphery of the nodule in the caecum. The same findings were obtained with Payer's patches. These structures have a close topographical relationship to the submucosal nerve plexuses. It is concluded that the Payer's patches play an important role in the uptake and presentation of antigens.

In the gut of the pig Payer's patches receive nerve fibres from the ganglia lying among the interfollicular region (8). In the cat the nerve fibres going to Payer's patches have their origin in the ganglionic submucosal plexus and the aganglionic mucosal plexus (13). From these fibres only a few nerve fibres were demonstrated reaching the periphery of the lymphatic follicles of the Payer's patches. The absence of the nerve fibres with direct contact with the centrally located lymphocytes can be replaced by the possible movement of the lymphoid cells in the space of the follicles and/or the movement of M-cells present in the epithelial lining of this region (8). As only a few nerve fibres were observed in the upper area of the lymphatic nodules and inside the follicles no nerve fibres were identified, our results showed that a structural association between enteric nerves and the compartments of Payer's patches exists in the internodular region where M-cells are not present.

From studies undertaken up to the present and also from our study it seems that most of the nerve fibres detected in the lymphatic organs are located in the periphery of the blood vessels in the submucosa and are related to their smooth muscle cells. From this disposition it can be proposed that they play a role in the flow of the blood cells, mainly lymphocytes, in the corresponding area (9). Occasional nerve fibres were also seen in T-areas among parenchymatous cells of human lymph nodes (15). According to Felten *et al.* (3) the presence of nerve fibres in lymphoid tissue suggests a potential structural link between the nervous and immune system that could be utilized for translating neural messages into chemical signals that interact with specific cellular elements in the immune system. The nerve fibres identified in the lymphatic tissue were described as noradrenergic, which according Felten *et al.* (4) was regional and specific and generally was directed into zones of T-lymphocytes and plasma cells, while avoiding nodular regions and zones of developing or maturing B-lymphocytes. Such a link was recorded in the lymph nodes (14), in the thymus (6, 18) and in the spleen (17, 21, 22).

The results we obtained showed that structural association between enteric nerves and the central compartments of lymphatic follicles of the ileum and caecum is absent and exists only at its periphery. For this reason it is concluded that a direct effect of the nervous system on the lymphoid tissue in this area is limited. The presence of nerve plexus inside the interfollicular regions rich in lymphoid cells in Payer's patches of the ileum and at the peripheric area of lymphatic nodules in the colon suggests an influence of the nervous system on the activity of mature lymphoid cells.

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LAPAROSCOPIC INTRAUTERINE INSEMINATION OF BITCHES

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ABSTRACT

This topic verifies the efficiency of laparoscopic intrauterine insemination and at the same time presents the potential of this method for the artificial insemination of bitches. Experiment has determined that sperm vaginal exposure is not required to achieve capacitation. Pregnancy after laparoscopic intrauterine insemination was present after 35 days in 75 % of the bitches. Slight local bleeding could persist at the site of penetration for insemination in the experimental animals for a few minutes. The bleeding time was relatively shorter and less intensive after the penetration of the uterine horn than the penetration of bifurcation and uterus. The cannula, which has a resistance to penetration, is not located directly at the center of the lumen and insemination is not successful. Only a smooth and easy insertion, which allows free movement of the cannula in lumen, is a correct productive method for insemination. Penetration into the uterine horn was easier than into the uterine body. Despite the fact that this method seems to be hard to perform, a skilled experienced surgeon will accomplish the whole procedure within fifteen minutes.

Key word: artifitial insemination; female dog; laparoscopy

INTRODUCTION

Interest in the artificial insemination of female dogs by fresh chilled, diluted and also deeply frozen semen has arisen especially in recent years (12, 13). In female dogs artificial insemination can also be successful after depositing semen intravaginally. However, results are better if the semen is deposited intrauterinally, above all, when using a defrost insemination dose. It is often difficult to penetrate the uterine cervix due to its anatomical peculiarities and presence of the middle dorsal fold (11, 15, 17). Despite these anatomical peculiarities Cobb already in 1959 (3) and later Lagers- tedt and Obel (10) had achieved a depositing of the contrast medium into the uterus by cervical catheterization.

The intrauterine insemination of female dogs can be achieved by four methods. Some authors have chosen the technique using a special catheter transcervically (1, 5, 6, 7, 12, 13, 14). This technique, however, requires a certain training, whereas in nervous or obese bitches it is impractical because of the difficult and almost impossible manipulation of the cervix through the abdomen.

Wilson (22) has described the technique of cervical catheterization by an endoscope as well as its limitations connected with vaginal length and width in a bitch.

The technique of intrauterine insemination laparoscopically is used in many countries (20). Its disadvantage is invasiveness and deep anaesthesia that can potentially interfere with the uterine motility and oocyte migration (9).

The work of Wildt (21), who achieved intrauterine catheterization to obtain the uterine fluid, preceded the first description of the laparoscopic intrauterine artificial insemination in bitches. Only in 1995 Silva *et al*. (18) is laparoscopic intrauterine insemination in female dogs reported. At the same time Silva and Verstegen (19) published the results comparing the efficiency of three various extendors of deeply frozen semen of dogs, introduced laparoscopically intrauterinally, on female dog fertility.

The aim of this study is to assert the efficiency of intrauterine insemination by laparoscope with insemination doses prepared from freshly collected ejaculates. Experiments have been designed to determine if canine sperm requires vaginal exposure to achieve capacitation too.

MATERIALS AND METHODS

Animals

For intrauterine artificial insemination we inseminated four bitches with fresh semen from one male dog. The age of the patients was between two and seven years, the dog and bitches were cross breeds German Shepherd. The animals were kept in clinical kennels and were fed commercial food (Pro Nature 26 Sousproduits de Boulanggerie L. B. Ltee, Canada), and were given water *ad libitum.*

Optic equipment and accessories

A laparoscope unit consisting of laparoscope MLW Germany, 180°, one centimetre in diameter, source of light, optic glass fiber, automatic flash, photographic apparatus with optic adaptor (Karl Zeiss, Germany) and Kodakchrome film, manual insufflator with regulator and T shaped pressure gage connected to CO_2 tang container.

Invasive and surgical instruments

For pneumoperitoneum we used a Veress needle. For insertion of the laparoscope we used a set of trocars and cannulas in a range of 0.5—1.5 centimetres with safety protection for the sharp tip. For manipulation with internal organs we used a rigid metal probe. For the grasping and fixation of internal organs and different parts or various instruments we used endopean forceps. For intrauterine artificial insemination we used a teflon cannula with a metal mandrin (Chiraflex, Slovakia).

Anaesthesia and preoperative preparations of the patient

With all the animals the same material and substances were used. Food was withheld for twenty-four hours and water for six hours before the operation.

Tranquilizer: Fifteen milligrams of diazepam i.m. (Apaurin) *pro toto*, fifteen minutes before introducing anasthesia. For sedation we used xylazin 2 mg.kg^{-1} b.w. i.m. (Rometar) and after ten minutes neuroleptic analgetic ketamin 10 mg.kg^{-1} b.w. i.m. (Narkamon) was injected.

The animals were fixed in a recumbent position with the head down and total body plane lying between thirty to forty° in relation to the ground. The surface between the pubic bone and four centimetres cranially to the *umbilicus* was shaved and scrubbed centimetres on either side.

Formation of pneumoperitoneum

The puncture of abdominal cavity with Veress needle was located two centimetres cranially to the pelvic bone and two centimetres laterally on each side (according to the convenience of surgeon) of *linea alba.* To confirm an intact bladder we aspirated with a syringe through the Veress needle. If urine was present, the Veress needle was reinserted. The Veress needle must penetrate all the abdominal wall layers to control subcutical, muscular or subperitoneal dislocation.

Determination of physiological stage of oestrus cycle in bitch

For observing the ovaries and for intrauterine artificial insemination by laparoscopy, the bitch was diagnosed for a specific oestrus cycle status by vaginal smear for exofoliative cytology using the Diff Quick staining method after Concannon and Digregorio (4).

Intrauterine artificial insemination by using laparoscopy

Fresh semen from a dog was produced by masturbation technique. Only a spermal fraction was collected. Sperm count was estimated by haemocytometric (Bürker's chamber) and microscopic methods, for motility. The dosage was one millilitre and was stored in warm bath water at 38°C, until the moment of insemination. A one centimetre diameter laparoscope with an optic angle of 180°, was introduced into the abdominal cavity on midline, one centimetre caudal to the *umbilicus*, using simple trocar with adjacent cannula. A second trocar (Surgiport) in diameter of 0.5 cm, was inserted on the right side, six to ten centimetres caudal to the umbilicus and two to three centimetres lateral to the *linea alba*. This was used for endo pean for the purpose of the fixation of specific uterine horn. A teflon cannula (Chiraflex) with a metal mandrin was introduced over uterine bifurcation. Under the control of the laparoscope, the metal mandrin invaded the specific site of insemination and the proper amount of semen was injected.

Table 1. Results of intrauterine artificial insemination by using a laparoscope in bitches with fresh semen

No. of bitch	Day of oestrus	Concentration of sperm.ml^{-1}	Motility %	Place of insemination	Sperm in vagina after 20 min	Sperm in vagina after 24 h
1	2	692.10^6	85	left horn cranially	++	+++
2	1	495.10^6	95	right horn	+	++
3	2	258.10^6	80	bifurcation	+	+
4	3	530.10^6	90	uterus	+	+

Legends:

\+ — rare appearance of sperm in many microscopic fields/fixed vaginal smear with Diff Quick

++ — rare appearance of sperm in one microscopic field/fixed vaginal smear with Diff Quick

+++ — 3 and more sperms in a single microscopic field/fixed vaginal smear with Diff Quick

RESULTS

We discovered that a cannula which has a resistance to penetration, is not located directly at the centre of the lumen and insemination is not successful. Only a smooth and easy insertion which allows free movement of cannula in lumen, is a correct productive method for insemination.

Penetration of the uterine horn is easier than the uterine body. Despite the fact that this method seems to be hard to perform, a skilled experienced surgeon will accomplish the whole procedure within fifteen minutes.

The third penetration is done with a No. 14 needle, which requires no treatment after withdrawing the needle.

The results of the insemination of the bitches (Tab. 1) were controlled by a vaginal smear which contained sperm. The sperm which appeared in vaginal smears, is a proof of a successful insemination with a 100 % accuracy.

In all four bitches, which were inseminated, a slight local bleeding would persist for a few minutes at the site of penetration for insemination. The bleeding time was relatively shorter and less intensive after penetration of the uterine horn than the penetration of bifurcation and uterus.

Three out of four bitches had signs of pregnancy thirty-five days after insemination by laparoscope. This indicates that canine sperms do not require vaginal exposure to achieve capacitation.

DISCUSSION

There are several situations when a direct depositing of sperm into the uterus can improve fertility in female dogs. Results obtained by the intravaginal insemination with deeply frozen semen are often unsatisfactory. From the work of Farstadt (5) it is evident that direct intrauterine insemination leads to significantly better results. In some individuals artificial insemination helps to overcome some anatomical obstacles or behaviour disorders contributing to infertility (8). Mating or artificial insemination in the case of late oestrus often leads to weak results, probably due to the endocrine changes, changes in the vagina, uterine cervix and uterus, which influences the passage of sperms (20). A direct depositing of an insemination dose into the uterus can overcome these problems.

The intrauterine insemination *per laparoscopiam* with fresh semen of the sperm concentration ranging from 258 to 692 × 10^6 in four bitches led to the pregnancy of three of them. The technique is relatively fast, because it lasts fifteen minutes on average. Bleeding after cannula removal stopped relatively quickly from the uterine horns. However, an intervention was not necessary. Neither fertility nor implantation was affected negatively by the insemination technique and manipulation with reproductive apparatus by endo-instruments. Tsutsui *et al*. (20) recorded the fast migration of sperms from the only intrauterine insemination site into the whole lumen of horns and uterus. From this it follows that only one intrauterine application is sufficient to gain conception.

The results of artificial insemination in female dogs with fresh semen *per vaginam* or intrauterinally range from 25 % to 89 % (2, 5, 12, 16). The success of the artificial insemination *per laparoscopiam* reported in this work is not unusual in comparison with the intravaginal results obtained by other authors during the optimal time of mating.

This work tests the effectiveness of laparoscopic intrauterine insemination, but also presents a potential of this technique for the artificial insemination of female dogs. Repeated insemination forty-eight hours after the first one is usually used in routine artificial insemination of female dogs. The technique *per laparoscopiam* facilitates the success of only one insemination dose. However, the optimal determination of the time of mating by vaginoscopy, vaginal cytology and evaluation of the levels of serum progesterone are preconditions of success. Laparoscopic intrauterine insemination can be used in the management of a mating programme in bitches, especially in the cases of infertility or insemination with deeply frozen semen.

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A PRELIMINARY CLASSIFICATION OF CAMEL TYPES OF THE AFDER ZONE OF SOMALI NATIONAL REGIONAL STATE, ETHIOPIA

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SUMMARY

The basic units of the sampling survey consist of fifteen households having more than ten camels in each of the five selected districts of the Afder zone in Ethiopia. Pretested questionnaires were administered and data were also obtained from researchers moving with the camel owners. The pastoralists classified their camels into *Gel-lab* and *Hoor* types. The *Gel-lab* type is known for its multipurpose use and said to be more resistance to drought and feed shortage whereas the *Hoor* type known for milk production with early maturity, faster growth, quicker loss of weight during dry season and more susceptibility to biting flies. The *Gel-lab* type can give tastier milk at shorter intervals (3.61 times/day) whereas *Hoor* type gives milk at relatively longer intervals (2.07 times/day). The *Hoor* type produced more daily yield of milk at early, peak and late lactation (2.90, 5.49 and 1.0 l.day^{-1}) than *Gel-lab* type (1.73, 3.45 and 0.50 l.day^{-1}, respectively). The *Gel-lab* had shorter lactation length (18.11 months) than the *Hoor* type (22.39 months). These two types living in a common geographical area for generations and reflecting such large physical and functional variations demand further research to classify utilise their attributes.

Key words: camel types; *Gel-lab* and *Hoor;* lactation length; milk off take

INTRODUCTION

The concept of breed can be applied to any group of animals, which are located in a geographical area and have some phenotypic character in common and are recognised by local people as a local type. In the absence of any written form of information on the breeds as in the case of developing countries, an important source of information that may prove valuable are the views of the farmers especially older farmers (4). The recent attempt to categorise multipurpose species like camels as specialised breeds such as milk, meat, transport, riding, and racing is too premature. Because of the nomadic way of life and interbreeding, there is relatively little differentiation into specialised breeds in camels.

In the Republic of Somalia, Mohamed (6) has classified camels into a Northern type and Southern type. The later type is further divided into *Hoor, Siifdaar* and *Eyddimo* type. There is no report to verify whether these types are found in bordering areas like the Afder zone of Ethiopia. Moreover, the traditional methods of classifying the camels of Ethiopia (5, 7) are restricted to easily the assessable area where camels are reared.

There is no report on the camel types of Afder zone, which is not easily accessible and where the camel plays most important role in lively hood of pastoral population. The purpose of this study is to classify camel types based on the indigenous knowledge of the pastoralists.

MATERIALS AND METHODS

Five out of the eight districts of Afder zone were deliberately selected. Fifteen households having more than ten camels in each of the five districts formed the basic unit of sampling. A systematically stratified random sampling technique was used. Structured pretested questionnaires were prepared and administered by the single visit formal survey method. However, responses and data were obtained moving with camel owners for months during the course of data collection. The long duration of stay with herders assisted in testing the repeatability of the responses. The further authenticity of responses was verified through community elders and district leaders.

The daily milk extracted was calculated based on traditional containers (Dhakal – 1.5 litres used during daytime milking and two types of glasses of 830 millilitres and 500 millilitres capacity used for selling the milk). Simple descriptive statistics were used.

RESULTS AND DISCUSSION

Production environment

The Afder zone is located in the south-east of Ethiopia and on the border of the Republic of Somalia. The altitude varies from 250 metres to 600 metres above sea level. The Afder zone is an arid ecological zone, with a low, unreliable, unevenly distributed rainfall pattern. The average rainfall varies from 150 millimetres to 350 millimetres. The temperature ranges from 25 °C to 40 °C during the dry season. When the temperature is high biting flies are very active and in abundance. The vegetation of the Afder zone includes scattered trees, annuals, perennial grasses, and legumes. Overgrazing and degradation of the soil are very common. Mainly pastoralism and on river beds agro-pastoralism are also practiced. Migration is common feature mainly within a clan's territory. However, during a very long dry period and drought, migration takes place regardless of national and international boundaries. The river bank areas are exposed to biting flies and areas away from river banks face a serious problem of water scarcity. Though the camels are only the means of transport, milk production is the main production goal. The main constraints of camel production are diseases, feed scarcity, water paucity and labor shortage (2).

Camel types

The pastoralists have classified their camels into two types (breeds) namely the *Gel-lab* type – multipurpose camels and *Hoor type known for milk production. The major subjective differences of their characteristics are depicted in Table 1 and some of the milk production traits are detailed in Table 2.*

The Gel-lab type of camel is said to be more resistant to drought and feed shortage. It may be because of its physical features such as long legs, which keep their body away from the hot ground and smaller body size.

It is quite interesting to note that the Gel-lab can give milk at short intervals. According to some of the pastoralists, the *Gel-lab* type of camels can be milked as many as three times in three hours period. On average they were milked 3.61 times in a day (Tab. 2). On physical examination of typical *Gel-lab* camels it was noticed that the udder is small in size. Since, in camels there is no milk reservoir beyond the teat cistern, there may be differences between these two types in the amount of glandular tissue and functioning of tubuloalveolar glands in milk secretion.

According to the camel owners' belief the quality and the taste of milk from *Gel-lab* is better than from the *Hoor*. It is natural that the animals that yield less milk will have more concentrated milk constituents and hence a better taste. The owners further reported that they normally used the milk of newly calved *Gel-lab* type camels, as a medicine to cure fractures and consumption of milk should be continued till the fracture wound is healed. It is possible that milk from this type may contain more minerals like calcium, which assists, in bone healing. However, the validity of this claim

Table 1. Comparison of some of the traits of camel types of Afder zone as perceived by the pastoralists

Sl. No.	Traits	Camel type *Hoor*	*Gel-lab*
1	Susceptibility to drought acceptance of change of milker	More	Less
2	Susceptibility to feed shortage	More	Less
3	Height	Shorter	Taller
4	Body size	Larger	Smaller
5	Maturity	Earlier	Later
6	Weight gain (growth)	Faster	Slower
7	Loss of weight during summer	Faster	Slower
8	Ability to let down milk at short intervals	Less	More
9	Ability to let down milk without a calf	Less	More
10	Taste of Milk	Less	More
11	Acceptance of foster calf	With difficulty	Easier
12	Acceptance of change of milker	With difficulty	Easier

Table 2. Some of milk production traits of camel types of the Afder zone based on the responses of pastoralists

Traits	Camel type *Hoor*	*Gel-lab*
Number of times milked per day	2.07 ± 0.71(74)	3.61 ± 0.84 (66)
Lactation length (month)	22.39 ± 5.66(74)	18.11 ± 5.62 (65)
Daily milk off take (l)		
Early lactation	2.90 ± 1.12 (72)	1.73 ± 0.47 (66)
Peak yield	5.49 ± 1.19 (69)	3.45 ± 1.05 (65)
Late lactation 0.50*	1.0* (72)	

Figures with in brackets are number of responses
* — S.D. is more than mean

remains to be investigated in terms of milk composition of newly calved camels.

Easy acceptance of a foster calf in the case of the death of its own calf and acceptance of change of milkers, as observed in the *Gel-lab* compared to the *Hoor,* may be peculiar to this type (breed). The explanation given by the herders is that the *Gel-lab* likes its calf more than the *Hoor* does and therefore accepts the foster calf in place of its own calf. Similar traits, besides the medicinal value and frequency of milking of the *Gel-lab,* were recorded for the *Siifdaar* type of camel of the Southern central regions, mainly on the lower Shebelle river banks of the Republic of Somalia (6).

The early maturity, faster growth and faster loss of weight during the dry season by the *Hoor* is typically characteristic of milk types of ruminants. This type is said to be very susceptible to biting flies unlike the *Gel-lab*. It is not known whether the perception of the susceptibility of the *Hoor* type and the resistance of the *Gel-lab* refers to the biting of flies or diseases such as trypanosomiasis caused by these vectors. The phenomena of susceptibility and resistance offers an unparallelled opportunity to improve camel production in vast areas of arid and semiarid regions dominated by these biting flies. As seen from the Table 2 the *Hoor* produced a greater daily yield at all stages of lactation. The milk off take reported here is the milk extracted for human consumption and should not be considered as the genetic potential of these animals. Moreover, early publication amply indicates that variation of milk yield from two to twenty litres for the *Hoor* and two to ten litres for *Siifdaar camels (6).*

These wide ranges suggest that potentially high yielding camels exist within the indigenous population among individual herds of farmers. Though the average milk off take is low compared to the measured yield as reported from other areas of Ethiopia (1, 3, 7), considering the harsh arid environment under which they produce, the yield is satisfactory and sustainable.

The long lactation length noticed for both types is no doubt an indication of the scarcity of feed and water, the effect of climatic factors and may be a management objective of pastoral dairying to have milk animals with extended lactation. Even under these conditions the *Hoor* had longer a lactation length of 22.39 months compared to the *Gel-lab* (18.11 months). However, Mohamed (6) reported a lactation length of eight to sixteen months and twelve months for the *Hoor* and *Gel-lab* respectively. *Hoor* camels are known as a milk type because of a higher daily milk off take and longer lactation length.

It is natural that geographically isolated livestock species may vary in their characteristics. But the consequences of living in a common geographical area for generations and reflecting such large physical and functional differences requires further research to classify and utilise their attributes. It is assumed that the present preliminary study based on the perception of pastorals will stimulate researchers to initiate a scientific characterisation of the camel types of arid and also inaccessible areas.

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THE STATUS OF MACRO- AND MICRO-ELEMENTS IN THE BLOOD SERUM, MILK, RUMEN FLUID, FAECES AND URINE IN A FARM WITH INCREASING MILK PRODUCTION

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ABSTRACT

From 1999 to 2003, on an East-Slovakian farm, we undertook a regular preventive diagnosis of dairy cows' diseases. Following the results of analyses, corrective measures were suggested to eliminate biochemical dysbalancies of the animals. The farm has an increasing milk production: in 1999 – 4200 litres, 2000 – 5100 litres, 2001 – 5800 litres, and in 2002 – 7079 litres. In this work we discuss the production, reproduction and health indices of the cows. We have paid special attention to selected macro- (Ca, P, Mg, Na, K) and micro-elements (Cu, Fe, Zn, Se) in the blood serum, milk, rumen fluid, faeces and urine of dairy cows selected by their reproduction cycle.

Key words: blood serum; dairy cows; faeces; macro-elements; micro-elements; milk; rumen fluid; urine

INTRODUCTION

Progress in cattle breeding in Slovakia should meet two basic requirements: 1. production of a sufficient quantity of good quality products for the national market-continuous increase in meat and milk consumption with such numbers of cattle, which permit a permanent improvement of the culture profile of the country and partly contribute to the alleviation of the social problem of unemployment; 2. regarding the globalisation of the world market and admission of Slovakia into the European Union. These ambitions require an intensification in cattle breeding, both for milk and meat production. Within this complex, the completion of programs of preventive diagnosing and health control guided by veterinary surgeon has an important role (3, 1).

MATERIAL AND METHODS

Farm characteristics: The farm has on average 365 dairy cows of the lowland black spotted breed with 75—92 % of the Holstein-Friesian breds in their genetic make-up; average body weight 550 kilograms. The cows are loosely housed with box resting places. Average milk production (by closed lactations, from January to December) reached in 1999 – 4200 litres, 2000 – 5100 litres, 2001 – 5800 litres, and in 2002 – 7079 litres of milk per cow.

The feeding system was organised in phases according to lactation stages: Foods in the form of mixed ration were distributed to corresponding groups of cows by a fodder carrier.

— *up to fourteen days after parturition*, ration the same as that up to one hundred days, however, food intake is approx. 60 % only, therefore the cows have free access to meadow hay;

— *up to one hundred days of lactation*, the ration balanced to daily milk yield of thirty-six litres. The ration consisted of twenty kilograms of maize silage, ten kilograms of haylage, three kilograms of alfalfa hay, nine kilograms of concentrated feed mixture DOP 1 (1.20 kilogram of soy, one kilogram of rape cakes, 300 grams of Megalac-saponified palm oil, 800 grams of Megapro – specially processed rape cakes and saponified palm oil, 300 grams of Vitamix S7CH-*Saccharomyces cerevisiae*, selenium, copper and in chelated form, maize, triticale, barley and wheat. Feeding salt was offered in licks. The ration dry matter was 21–22 kilograms and crude fibre in dry matter 17 %;

— *up to 200 days of lactation*, the ration was balanced to daily milk yield twenty-five litres and consisted of twenty kilograms of maize silage, ten kilograms of haylage, three kilograms of hay and six kilograms of feed mixture DOP2. The ration dry matter was nineteen kilograms and crude fibre in dry matter 18 %;

— *up to 300 days of lactation*, the ration was balanced to daily milk yield fifteen litres and consisted of sixteen kilograms of maize silage, seven kilograms of haylage, three kilograms of hay and three kilograms of feed mixture DOP2. The ration dry matter was fourteen kilograms and crude fibre in dry matter 20 %;

— *in dried cows (up to 3 weeks before parturition)* the ration was balanced to daily milk yield seven to eight litres and consisted of six kilograms of maize silage, seven kilograms of haylage, five kilograms of alfalfa hay and 250 grams of Vitamix S1CH. The ration dry matter was ten kilograms and crude fibre in dry matter 25 %;

— *in cows going to parturition (from three weeks before calving),* the ration was balanced to daily milk yield eighteen litres and consisted of ten kilograms of maize silage, ten kilograms of haylage, five kilograms of meadow hay, three kilograms of feed mixture for pre-partal cows (with up to 400 grams of Protemix containing chelated selenium, zinc, magnesium and copper). The ration dry matter was twelve kilograms and crude fibre in dry matter 24 %. To prevent postparturient paresis, the ration contained eighty grams of calcium and fifty grams of phosphorus.

Health disorders: From 1999 to 2000 an increased occurrence of puerperal disorders was recorded (retained placenta, endometritis, etc.) resulting in poor reproduction indices (service period — SP 180 days, insemination index over 3.0). A greater number of cows died or the cows were slaughtered (culling rate up to 40 %). Other findings included increased numbers of somatic cells in pooled milk samples (600–700 000) and locomotion disorders (pododermatits, Rusterholz's ulcer). From 2001 to 2002 there was some improvement in the aforementioned indices, which was shown in the evaluation of January 2003: Reproduction indices were as follows: service period — SP 127 days; insemination interval eighty days. Among reproduction disorders, retained placenta and endometritis were observed in 9.8 and 19.6 % of the cows, respectively. Disorders of the locomotion system consisted of acute and chronic aseptic laminitis *(pododermatitis circ. aseptica acuta et chronica)* and Rusterhoz's ulcers *(ulcus Rusterholzi)* (11.8 %), inflammation of digital *(dermatitis digitalis)* and interdigital *(dermatitis interdigitalis)* skin (59.7 %), and foot necrobacillosis (3 %) due to stall infection. The frequency of mastitis was 4.8 % (in the past, the main cause being trauma, presently the cows are dehorned), and the numbers of somatic cells in pooled milk samples ranged around 350 000. The culling rate reached 26 % due to reproductive disorders (49.6 %), low milk yield and old age (28.9 %), postparturient health disorders (11.6 %), and locomotion disorders (9.9 %). Seven cows (1,9 %) suddenly died, mostly due to liver failure.

Table 1. Blood serum concentrations of macro- and micro-elements in dried cows, cows after calving, and lactating cows (Ca, P, Mg, Na, K-mmol.l^{-1}; Cu, Fe, Zn, Se-µmol.l^{-1})

dried cows		**Ca**	**P**	**Mg**	**Na**	**K**	**Cu**	**Fe**	**Zn**	**Se**
Sampling I	$\bar{x}$	2.38	2.22	0.99	146.33	5.33	11.02	15.22	14.06	0.373
	sd	0.14	0.20	0.12	1.34	0.41	1.44	1.16	2.20	0.044
Sampling II	$\bar{x}$	2.61	1.75	0.89	147.00	4.55	6.16	16.86	12.87	0.301
	sd	0.08	0.15	0.07	1.53	0.41	1.06	2.03	1.43	0.056
Sampling III	$\bar{x}$	2.06	1.65	0.91	141.37	4.38	9.24	16.89	10.12	0.281
	sd	0.12	0.29	0.07	1.18	0.40	1.00	2.05	1.13	0.055
Sampling IV	$\bar{x}$	2.60	1.98	0.76	143.08	4.58	11.80	14.91	9.99	0.306
	sd	0.16	0.23	0.04	2.27	0.43	1.71	1.63	1.70	0.036
cows after calving		**Ca**	**P**	**Mg**	**Na**	**K**	**Cu**	**Fe**	**Zn**	**Se**
Sampling I	$\bar{x}$	2.44	1.58	0.89	147.50	4.24	11.40	12.98	13.76	0.358
	sd	0.11	0.26	0.08	1.71	0.40	1.74	0.86	2.42	0.043
Sampling II	$\bar{x}$	2.38	1.73	0.75	146.00	4.62	6.42	14.47	11.34	0.308
	sd	0.12	0.29	0.07	1.91	0.43	0.84	1.59	1.03	0.044
Sampling III	$\bar{x}$	2.06	2.06	0.77	143.00	4.90	11.40	15.43	8.69	0.288
	sd	0.13	0.23	0.07	3.29	0.42	2.66	2.82	1.15	0.045
Sampling IV	$\bar{x}$	2.39	1.93	0.72	142.00	4.29	12.06	14.92	11.20	0.284
	sd	0.15	0.31	0.07	1.89	0.45	1.77	1.22	1.42	0.042
lactating cows		**Ca**	**P**	**Mg**	**Na**	**K**	**Cu**	**Fe**	**Zn**	**Se**
Sampling I	$\bar{x}$	2.30	1.85	1.01	140.83	4.53	11.80	14.77	14.91	0.392
	sd	0.10	0.27	0.06	4.30	0.15	1.82	1.44	1.06	0.059
Sampling II	$\bar{x}$	2.40	1.81	0.91	145.83	4.77	7.08	13.43	12.74	0.289
	sd	0.13	0.52	0.09	3.34	0.36	1.02	1.03	0.85	0.039
Sampling III	$\bar{x}$	2.15	1.70	0.88	143.37	4.82	13.27	19.13	9.54	0.309
	sd	0.13	0.44	0.09	1.92	0.55	1.36	2.38	1.00	0.055
Sampling IV	$\bar{x}$	2.54	2.03	0.76	142.58	4.65	10.16	14.70	11.58	0.277
	sd	0.16	0.29	0.05	2.23	0.32	1.99	2.24	1.21	
0.051										

Table 2. Milk concentrations of macro- and micro-elements in dried cows, cows after calving, and lactating cows (mg.l^{-1})

cows after calving		Ca	P	Mg	Na	K	Cu	Fe	Zn	Se
Sampling I	$\bar{x}$	1108.33	395.84	119.50	795.00	1125.00	0.18	0.46	3.79	0.007
	sd	95.82	33.14	16.15	440.41	229.26	0.11	0.09	1.03	0.001
Sampling II	$\bar{x}$	1122.33	433.32	102.50	654.17	1466.83	0.06	0.43	3.20	0.008
	sd	87.81	20.44	12.79	171.77	154.04	0.02	0.22	1.04	0.002
Sampling III	$\bar{x}$	1215.71	429.91	109.71	741.43	1257.14	0.63	0.25	3.68	0.007
	sd	124.94	51.10	9.50	398.93	286.81	0.21	0.10	0.79	0.001
Sampling IV	$\bar{x}$	982.66	382.52	101.00	778.33	1293.33	0.30	0.36	3.45	0.007
	sd	94.33	23.23	12.72	305.05	133.06	0.14	0.14	0.35	0.001
lactating cows		**Ca**	**P**	**Mg**	**Na**	**K**	**Cu**	**Fe**	**Zn**	**Se**
Sampling I	$\bar{x}$	951.67	410.71	109.67	805.00	1146	0.11	0.29	3.20	0.011
	sd	192.48	21.68	7.36	408.85	225	0.06	0.07	1.10	0.002
Sampling II	$\bar{x}$	928.67	386.24	96.00	847.50	1187	0.07	0.24	2.81	0.007
	sd	240.82	62.25	16.75	351.73	400	0.02	0.09	0.91	0.001
Sampling III	$\bar{x}$	965.00	380.97	94.75	906.25	1180	0.54	0.23	2.82	0.007
	sd	164.70	21.68	9.98	271.11	252	0.09	0.10	0.78	0.001
Sampling IV	$\bar{x}$	866.08	384.38	98.00	936.66	1185	0.29	0.29	2.94	0.007
	sd	74.44	33.45	7.22	314.73	175.57	0.11	0.14	0.59	0.001

Table 3. Rumen fluid concentrations of macro- and micro-elements in dried cows, cows after calving, and lactating cows (mg.l^{-1})

dried cows		Ca	P	Mg	Na	K	Cu	Fe	Zn	Se
Sampling II	$\bar{x}$	222.67	90.98	76.50	2165	602	0.97	8.77	6.32	0.011
	sd	41.29	58.48	14.72	204	115	0.48	2.48	1.51	0.001
Sampling III	$\bar{x}$	253.91	634.36	107.03	1959	676	0.49	5.05	3.66	0.011
	sd	51.37	384.01	23.63	315	184	0.16	2.20	0.91	0.001
Sampling IV	$\bar{x}$	208.50	542.03	101.87	2162	639	0.37	5.22	4.60	0.011
	sd	42.36	291.74	24.80	216	99	0.12	1.98	1.34	0.001
cows after calving		**Ca**	**P**	**Mg**	**Na**	**K**	**Cu**	**Fe**	**Zn**	**Se**
Sampling II	$\bar{x}$	124.83	57.82	76.33	1846	562	0.30	3.23	3.42	0.013
	sd	51.54	49.45	29.04	279	146	0.12	2.69	0.95	0.002
Sampling III	$\bar{x}$	172.64	1010.5	79.17	1692	679	0.38	5.57	3.56	0.010
	sd	47.59	433.01	20.12	290	170	0.13	3.34	1.03	0.001
Sampling IV	$\bar{x}$	220.00	502.21	70.00	1485	475	0.60	5.20	6.00	0.010
	sd	62.30	265.18	22.41	276	158	0.21	2.65	1.24	0.002
lactating cows		**Ca**	**P**	**Mg**	**Na**	**K**	**Cu**	**Fe**	**Zn**	**Se**
Sampling II	$\bar{x}$	214.17	118.98	106.50	1721	710	0.92	5.78	6.10	0.012
	sd	49.12	97.62	11.03	205	71	0.61	1.71	1.05	0.001
Sampling III	$\bar{x}$	193.91	978.24	97.97	4085	751	0.75	6.58	4.76	0.010
	sd	73.54	497.29	16.43	5263	178	0.24	2.38	1.44	0.002
Sampling IV	$\bar{x}$	146.25	558.62	95.62	1728	662	0.65	3.90	4.05	0.009
	sd	62.14	236.80	15.85	192	74	0.19	1.36	1.34	0.002

Table 4. Faeces concentrations of macro- and micro-elements in dried cows, cows after calving, and lactating cows (mg.kg^{-1})

dried cows	Ca	P	Mg	Na	K	Cu	Fe	Zn	Se	
Sampling I	$\bar{x}$	1862	590	787	582	1486	6.75	123	55	0.053
	sd	588	255	248	309	211	2.66	25	18	0.013
Sampling II	$\bar{x}$	2568	985	784	599	1092	4.76	114	48	0.051
	sd	368	392	111	187	187	0.97	16	5	0.011
Sampling III	$\bar{x}$	1616	2566	820	386	1022	5.93	106	51	0.041
	sd	392	1179	241	87	257	1.44	41	13	0.007
Sampling IV	$\bar{x}$	2412	1489	711	420	1193	5.94	128	72	0.055
	sd	297	273	232	83	161	1.52	24	19	0.018
cows after calving		**Ca**	**P**	**Mg**	**Na**	**K**	**Cu**	**Fe**	**Zn**	**Se**
Sampling I	$\bar{x}$	883	432	589	194	1800	3.09	177	40	0.059
	sd	199	176	91	56	486	0.58	141	6	0.028
Sampling II	$\bar{x}$	3222	1253	973	480	1218	6.76	135	69	0.059
	sd	1373	252	345	138	414	2.96	57	22	0.010
Sampling III	$\bar{x}$	2467	2415	1408	365	864	4.56	102	67	0.046
	sd	774	843	463	136	129	1.19	29	14	0.012
Sampling IV	$\bar{x}$	1989	1862	823	372	823	4.29	118	42	0.052
	sd	752	374	248	125	119	1.21	23	7	0.010
lactating cows		**Ca**	**P**	**Mg**	**Na**	**K**	**Cu**	**Fe**	**Zn**	**Se**
Sampling I	$\bar{x}$	1015	823	647	220	1486	4.84	141	49	0.069
	sd	273	315	56	218	244	0.89	56	4	0.032
Sampling II	$\bar{x}$	2418	707	912	285	962	5.56	108	59	0.053
	sd	565	371	145	163	209	1.69	14	7	0.015
Sampling III	$\bar{x}$	2740	1234	1019	493	1077	6.05	137	45	0.047
	sd	914	391	350	137	225	1.26	31	12	0.021
Sampling IV	$\bar{x}$	3064	1521	1668	415	1024	5.24	96	64	0.048
	sd	815	318	294	132	197	1.58	13	18	0.009

Collection of samples: Samples for the metabolic profile test were collected from three groups of dairy cows (each group of six animals, February 2000, March 2001, January 2002, January 2003): 1st group — dry cows (one month before calving); 2nd group — cows after parturition (first 15 days); 3rd group — lactating cows (two months after parturition).

Laboratory analyses: Examination of blood serum: Ca, Mg, Na, K – diluted 1:100 + 5 % solution of La_2O_3; Cu, Fe, Zn – measured after deproteinization (trichloracetic acid). ***Examination of milk and urine:*** calcium, magnesium, sodium and potassium, — diluted 1:100 + 5 % solution of La_2O_3; copper, iron and zinc — measured after deproteinization (trichloracetic acid). Examination of rumen fluid and faeces: Wet mineralisation (HNO_3 1:1; 30 % H_2O_2) in microwave owen MLS 1200; calcium, magnesium, sodium and potassium — diluted 1:25; copper, iron and zinc — directly measured in mineralisate.

Measurement: Flame absorption spectrophotometry — A Analyst 100; selenium analysed in graphite cuvette by flameless method — AAS 4100 ZL; analyses of phosphorus in the blood serum, milk and urine — spectrophotometric analyser Alyze, Lisabo, bio Mérieux; analyses of phosphorus in the rumen fluid and faeces method by Urbach Rabe. Statistical analyses were performed by the computer program Statgraphics vers. 4.0.

RESULTS

The concentrations of macro- and micro-elements in the blood serum, milk, rumen fluid, urine, and faeces in the corresponding groups of cows are presented in Tables 1—5.

DISCUSSION

Concentrations of selected macro- and micro-elements in the blood serum: reference values in adult cattle range in: calcium 2.25—2.80; phosphorus 1.62—2.26;

Table 5. Urine concentrations of macro- and micro-elements in dried cows, cows after calving, and lactating cows (mg.l^{-1})

dried cows		Ca	P	Mg	Na	K	Cu	Fe	Zn	Se
Sampling I	$\bar{x}$	53.33	65.35	661.67	110.33	11486	0.20	0.82	0.56	0.008
	sd	45.46	59.46	361.77	171.10	6739	0.16	0.14	0.13	0.001
Sampling II	$\bar{x}$	122.33	28.18	398.50	589.33	16550	0.13	0.51	0.08	0.007
	sd	75.85	9.29	103.41	407.97	3819	0.04	0.16	0.04	0.001
Sampling III	$\bar{x}$	20.33	28.00	141.17	49.83	4866	0.38	0.38	0.15	0.006
	sd	7.39	12.38	65.20	39.64	2655	0.23	0.11	0.05	0.0008
Sampling IV	$\bar{x}$	50.58	17.34	401.66	315.58	10475	0.23	0.30	0.30	0.007
	sd	20.65	4.95	138.94	265.70	2620	0.08	0.08	0.11	0.001
cows after calving		**Ca**	**P**	**Mg**	**Na**	**K**	**Cu**	**Fe**	**Zn**	**Se**
Sampling I	$\bar{x}$	19.33	40.26	291.67	19.33	12585	0.07	0.68	0.42	0.008
	sd	12.15	35.61	227.41	11.34	5613	0.02	0.20	0.12	0.001
Sampling II	$\bar{x}$	56.83	162.30	160.00	933.33	12416	0.28	0.48	0.09	0.007
	sd	70.95	107.16	73.21	948.06	6215	0.15	0.16	0.03	0.001
Sampling III	$\bar{x}$	19.20	83.62	123.20	112.80	6000	0.40	0.27	0.13	0.008
	sd	4.62	56.06	59.02	147.11	2376	0.27	0.05	0.04	0.001
Sampling IV	$\bar{x}$	86.33	38.71	244.16	324.16	7400	0.27	0.25	0.32	0.008
	sd	41.05	25.08	128.70	422.11	2954	0.09	0.09	0.12	0.001
lactating cows		**Ca**	**P**	**Mg**	**Na**	**K**	**Cu**	**Fe**	**Zn**	**Se**
Sampling I	$\bar{x}$	16.80	49.86	568.00	329.60	13820	0.07	0.93	0.39	0.008
	sd	4.53	34.69	169.52	359.77	1758	0.02	0.04	0.11	0.001
Sampling II	$\bar{x}$	14.67	63.18	292.00	986.83	15266	0.14	0.21	0.08	0.006
	sd	5.76	52.03	146.85	916.58	5678	0.12	0.12	0.04	0.001
Sampling III	$\bar{x}$	19.00	45.84	503.00	417.38	9031	0.38	0.25	0.17	0.008
	sd	3.87	6.81	23.35	108.37	5330	0.18	0.18	0.06	0.001
Sampling IV	$\bar{x}$	52.41	29.42	596.66	626.33	9816	0.30	0.37	0.41	0.007
	sd	29.78	16.41	286.11	357.62	3169	0.20	0.11	0.15	0.001

magnesium 0.78—1.23; sodium 136.0—150.0; potassium 4.00—5.50 mmol.l^{-1}; copper 12.6—18.9; iron 21.5—32.7; zinc 12.2—30.0; selenium 0.5—1.90 mmol. l^{-1} (4). When compared with the aforementioned data, in our work we recorded marked hypocupraemia, sideropenia, hypozincaemia, and hyposelenioaemia in all the groups of cows.

Concentrations of selected macro- and micro-elements in the milk: according to Jenness (1) bovine milk contains calcium 1206.40; phosphorus 690.71; magnesium 123.99; sodium 579.37; potassium 1380.30; copper 0.010—1.200; iron 0.100—2.400; zinc about 4.0; selenium 0.004—1.20 mg.l^{-1}. In our results, the most marked differences, compared with the reference values, were found in phosphorus concentrations, which were substantially lower (380.97—433 mg.l^{-1}). Concentrations of calcium and phosphorus, calculated to dry matter, are 880—1010 and 810—1080 mg.l^{-1}, respectively (6). The average contents of calcium in individual samples of bovine milk during the summer period is about 1116 mg.l^{-1} (959—1362 mg. l^{-1}) and during the winter time 1033—1414 mg.l^{-1} (5).

Concentrations of selected macro- and micro-elements in the rumen fluid: according to Williams *et al.* (6), rumen fluid phosphorus concentrations vary from 4 900 to 6 100 mg.kg^{-1} of dry matter, depending on the diet. With regard to other indices, we found no data in the available literature.

Concentrations of selected macro- and micro-elements in the faeces: available literature data are scarce. Wil-liams *et al.* (6) reported phosphorus concentrations from 3 500 to 5 200 mg.kg^{-1} of dry matter.

Concentrations of selected macro- and micro-elements in the urine: reference values range in: calcium 4.80—60.12; phosphorus 9.91—160.13; sodium 459.82—1839.28; potassium 5474.14—12512.32 mg. l^{-1} (4). Compared with our results we found deviations in sodium concentrations (lower values) and potassium (both higher and lower values).

The aforementioned data indicate a need for more detailed investigation into reference values for the materials examined.

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INFECTIOUS KERATOCONJUNCTIVITIS IN SHEEP

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ABSTRACT

We present the occurrence of infectious keratoconjunctivitis in sheep in the East Slovakian region. We describe case history data, clinical (particularly on the eyes) and laboratory findings (blood examination — haematological, macro- and micro-mineral profiles, serological, bacteriological, and coprological examinations). The most important findings are the culturing of *Mycoplasma* sp. and *Branhamella ovis* from eye swabs and seropositivity for *Chlamydia.* Following tests for susceptibility to antibiotics *(Branhamella ovis),* sheep in the clinic and farm were treated with the eye ointment Ophtalmo-Chloramphenicol (Léčiva-Prague). To complete the information about this disease, we present this disease complex in a conventional structure based on published literature.

Key words: *Chlamyophila psittaci;* keratoconjunctivitis; *Mycoplasma conjunctivae;* sheep

INTRODUCTION

In the available literature we find this disease named as contagious keratoconjunctivitis, contagious ophtalmia, inclusion body keratonconjunctivitis, pinkeye, heather blindness, snow blindness.

Occurrence: Infectious keratoconjunctivitis occurs in sheep throughout the world. The majority of cases are dealt by flock-masters without veterinary assistance. All ages of sheep may be affected, and cases occur at all seasons of the year.

Economical importance: While the disease appears painful and distressing for the sheep affected, it is usually of little economic consequence, except for the cost of treatment. Pregnancy toxaemia and agalactia are possible sequels for heavily pregnant ewes that cannot feed properly through blindness.

Aetiology and pathogenesis: Two infectious agents, *Chlamyophila psittaci (Chlamydia psittaci)* and *Mycoplasma conjunctivae,* are considered to be primary pathogens. Both agents have been isolated from the eyes of infected sheep in parts of the world, including Slovakia (3). However, *M. conjunctivae* is more frequently isolated than *C. psittaci.* The introduction of sheep with mild or non-apparent latent infection is the principal means of transmission of keratoconjunctivitis between flocks. The disease is readily spread within flocks when the sheep are in close contact, as when feeding at troughs, close yarding, transportation or when ewes are herded together for mating. The occurrence of conjunctivitis, pneumonia and polyarthritis in lambs is directly related to chlamydial infections in ewes (intrauterine infections). Outbreaks occur at all seasons of the year but can be more obvious in the autumn and winter, when sheep are gathered — mating, feeding or housing. Cases in adult sheep in the summer may be more severe, exacerbated due to the greater intensity of sunlight.

Bacteria: Many different genera of bacteria have been isolated from both clinically normal and affected eyes of sheep. However, *Branhamella ovis* and *Escherichia coli* occur more frequently in affected eyes. In one study, *Staphylococcus aureus* occurred more frequently in mildly affected eyes. These bacteria may monopolise the primary lesion caused by *Chlamydia* or myoplasmas and cause a more severe form of keratocojunctivitis (2).

MATERIAL AND METHODS

On 3rd October 2002, three adult ewes (3 years) were hospitalised in our Second Clinic for Internal Diseases. The animals from a private farm in the Košice rural district were destined for clinical and laboratory examinations aimed at reaching a

Figure 1. Numerous thistles in wool over the whole body

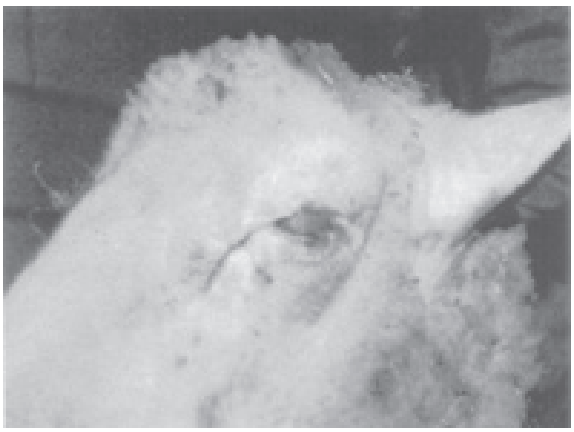

Figure 2. Incomplete closure of the eyelids on the affected eye

Figure 3. Closed eyelids, lacrimation

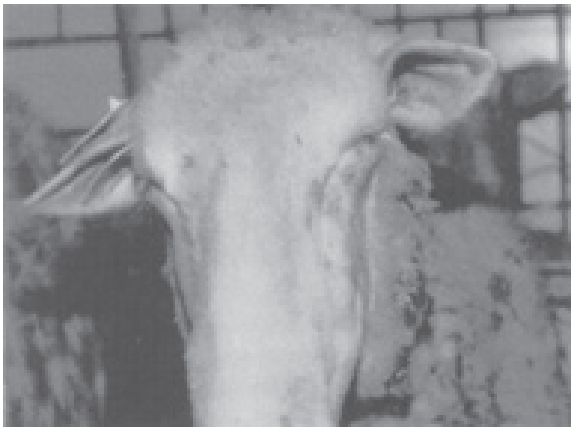

Figure 4. Bilateral closure of the eyelids

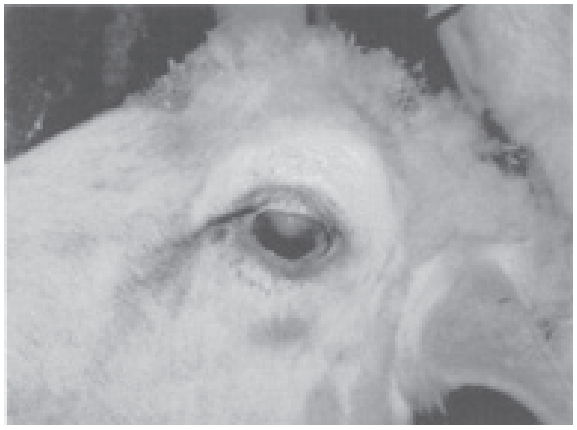

Figure 5. Mild corneal haziness

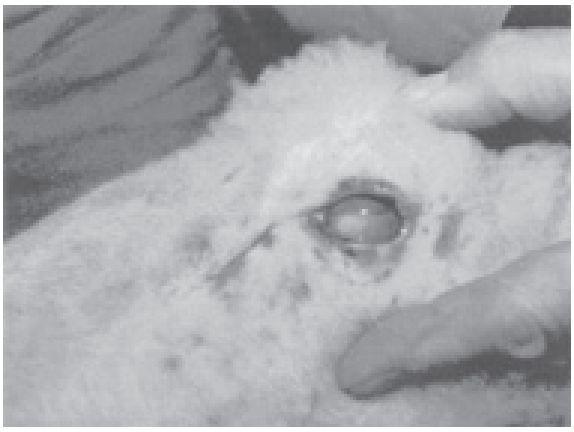

Figure 6. More marked corneal haziness

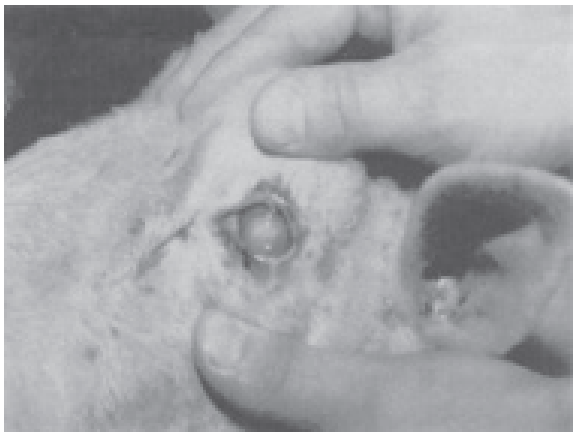

Figure 7. Severe diffuse corneal haziness

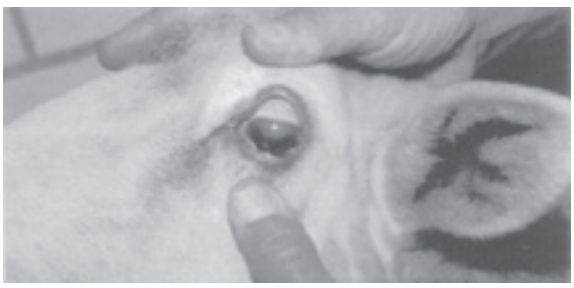

Figure 8. Marked injection of scleral vessels

proposal for therapeutic and preventive measures. The farmer and veterinary surgeon gave us the following case history data:

Sheep (ewes and young females — *mated*) were pastured near the village in unmown, weedy pasture contained thistle and high grass. During the preceding two weeks (September), many sheep showed lacrimation, closed eyes, purulent discharge and corneal haziness. Among 350 sheep in the flock, 100 animals were affected. Rams, which were housed during the night and pastured together with sheep (free mating) were not affected. There was a suspicion of a traumatic cause of the eye lesions.

Following an agreement with the farmer, the three ewes were hospitalised in our clinic from 3rd to 9th October 2002.

RESULTS

General clinical examinations revealed the following state of the animals:

1. A number of thistles in their wool over the whole body (Fig. 1).

2. Various degree of pathological changes on the eyes and surroundings:

— *in the first sheep:* moderate lacrimation from the right eye, conjunctivitis, mild injection of scleral vessels, mild diffuse haziness of the right eye cornea;

— *in the second sheep:* bilateral lacrimation, hyperaemic conjunctivae, mild injection of scleral vessels, mild diffuse corneal haziness central and ventral more marked local cloudiness, scleral hyperaemia (Figs. 2, 5);

— *in the third sheep:* Both eyes closed, increased lacrimation (Fig. 3), dried discharge near medial eye corners, swelling of eyelids, marked vascularisation of the sclera, right eye cornea markedly turbid with altered surface integrity in the middle, minimum response to light and hand movement, diffuse cloudiness of the left eye cornea, response to light and hand movement retained (Figs. 4, 6, 7, 8).

During hospitalisation, the sheep were examined in detail and biological material — blood, faeces, eye swabs — was analysed in the laboratory. Laboratory examinations revealed the following findings:

Leukocytosis (14.0 and 26.2 $G.l^{-1}$ in sheep with more severe eye findings); anaemia in one sheep with mild eye changes (HB 7.3 $g.dl^{-1}$, PCV 0.24 $l.l^{-1}$, Er 6.18 $T.l^{-1}$).

In the blood serum macro- and micromineral profiles, the most severely affected sheep showed hypophosphataemia (P — 1.15 $mmol.l^{-1}$), hypomagnesaemia (Mg — 0.22 $mmol.l^{-1}$), hyponatraemia (129 $mmol.l^{-1}$), sideropenia (Fe — 15.2 $\mu mol.l^{-1}$), hypocupreaemia (Cu — 7.87 $\mu mol.l^{-1}$), and hypozincaemia (Zn — 6.11 $\mu mol.l^{-1}$) — suggested relation to decreased food intake.

Blood serum concentrations of total bilirubin ranged near or even exceeded the upper physiological limit (from 6.49 to 7.86 $\mu mol.l^{-1}$), which was probably related to the presence of eggs of *Dicrocoelium dendriticum* in the faeces.

In all the sheep, in the faeces, the following eggs or oocysts were found: *Dicrocoelium dendriticum, Trichuris ovis,* and *Eimeria* spp.

From three samples of eye swabs (hospitalised ewes), in one *Mycoplasma* sp. was cultured. In seven eye swabs (three hospitalised ewes and four ewes in the farm) *Branhamella ovis* was cultured. Serological tests (CFT complement fixation test) of the hospitalised ewes revealed titres against *Chlamydia* 1:256 (ewe with mild keratoconjunctivitis) and 1:64 and 1:32 (remaining two ewes).

Following tests for susceptibility to antibiotics *(Branhamella ovis),* sheep in the clinic and farm were treated with the eye ointment Ophtalmo-Chloramphenicol into the conjunctival sac for about six days. In clinical conditions, the ewe with mild keratoconjunctivitis showed a marked improvement in the health state. Among ewes in the farm, seven animals showed persistent eye lesions (mild haziness), however, vision was retained.

DISCUSSION

All descriptions of the clinical appearance of ovine keratoconjunctivitis are similar, regardless of the causal agent identified. One or both eyes may be affected. In general, four stages of ovine keratoconjunctivitis are recognised:

Stage I (first): Hyperaemia of the palpebral and the bulbar conjunctival vessels, serous lacrimation, increased blinking and blepharospasm. The corneoscleral junction may show congestion in preparation for vascular migration (pannus) into the cornea. Most cases do not progress beyond this stage, but regress spontaneously.

Stage II (second): Continuation of stage I, characterised by corneal inflammation with blood vessels and pannus spreading from the corneoscleral margin. Early keratitis causes great irritation and, consequently, the blepharospasm and lacrimation are more obvious. Again spontaneous regression also occurs.

Stage III (third): Progresses from stage II, to a more mucopurulent keratitis with extension of migrating blood vessels and a more purulent lachrymal discharge. Sometimes shallow corneal ulceration is apparent, which affects vision. These cases are very obvious and usually receive treatment.

Stage IV (fourth): A corneal ulcer develops and vision is lost. Pus may be present in the anterior chamber of the eye (hypopyon). These cases are slow to resolve, even with treatment. Corneal scarring may persist permanently following resolution.

Lymphoid follicle hyperplasia causes the conjunctiva and third eyelid to appear granular and nodular. Frequently, the disease is less severe in lambs and most cases do not progress through all the stages described. Corneal ulceration also is rare in lambs.

Relapses in treated and naturally recovered animals commonly occur in keratoconjunctivitis associated with either *Chlamydia* or mycoplasmas. In the USA, polyarthritis may affect up to 85 percent of feedlot lambs with *Chlamydia*-associated conjunctivitis (4).

Necropsy findings: In the acute stages of *M. conjunctivae* infection, the conjunctiva and cornea are infiltrated with large numbers of neutrophils and small numbers of plasma cells and lymphocytes. In one study, the neutrophil count rose one day after inoculation to a peak eight days after inoculation. It then decreased from day 13 to return to negligible values after day 25. Samples of conjunctival cells can be taken readily by scraping the conjunctival fornix with a rounded, blunt spatula after local anaesthesia by the installation of 0.4 % oxybuprocaine hydrochloride. The cells are then smeared onto slides, fixed and stained by the Giemsa method. The presence of large numbers of uni-, bi- or tripolar bodies of approximately 250—1100 μm in diameter closely associated with epithelial cells is typical of *M. conjunctivae*. These bodies specifically fluoresce when antiserum to *M. conjunctivae* is used.

Diagnosis and differential diagnosis: Infections are by far the most common cause of keratoconjunctivitis in sheep. The clinical diagnosis is readily made after foreign bodies and entropions are eliminated. The differential diagnoses include pine or cobalt deficiency, pasteurellosis, bluetongue, and other eye diseases:

Bright blindness — a possible consequence of prolonged ingestion of bracken *(Pteridium aquilinum)* is bright blindness, a progressive retinal degeneration. Animals over two years of age are most commonly affected and incidence is highest in animals three to four years old.

Ophthalmitis associated with *Listeria monocytogenes* — an ophthalmitis preceded by conjunctivitis due to *Listeria monocytogenes* — conjunctivitis followed by ophtalmitis has been described in cattle and sheep fed baled silage from hoppers or ring feeders. Clinical examination revealed blepharospasm, cloudiness of the cornea and a swollen, folded iris and pupillary constriction. In some sheep, floccular material was present in the anterior chamber of the eye and a catarrhal conjunctivitis. *L. monocytogenes* was isolated from conjunctival swabs. The response to topical treatment was poor but, when combined with parenteral ampicillin, resolution was obtained within 2 weeks. The condition may be prevented by reducing ocular contact with silage through feeding in troughs.

Parasitic keratoconjunctivitis — the causal organism is *Thelazia californiensis,* the nematode eye-worm, is sometimes found in the conjunctival sac or on the surface of the cornea and may cause discomfort and contribute to the development of conjunctivitis. The life cycle is indirect, with flies (*Musca* spp.) acting as intermediate hosts. They deposit the larvae of the worms in the conjunctival sac when feeding round the eyes. Systemic anthelmintics are likely to be effective in removing these nematode parasites.

Developmental abnormalities — entropion (inversion of eyelid) — congenital entropion is a common cause of traumatic keratitis in neonatal lambs. Examination reveals the lower lid rolled inwards, causing the hairs to rub on the surface of the cornea. There is a secondary keratoconjunctivitis, epiphora and squinting. The severity is variable. Mild cases can be treated by a subconjunctival injection of an antibiotic such as a penicillin, which everts the lid. Michel clips can be used to staple a vertical fold of the lower eyelid in more severe cases. In the most severe cases, surgical removal of a strip of skin from the lower eyelid is advisable. The operation requires anaesthesia and disinfection of the site prior to surgery. Entropion is an inherited condition, and rams that produce offspring with entropion should not be used for further breeding.

Palpebral coloboma — this is a rare condition to which Manx Laughton sheep that possess four horns appear to be most susceptible. The upper eyelids are usually affected by coloboma (splitting). The mode of inheritance is unclear.

Diagnosis is made usually according to the clinical signs and response to specific treatment. Etiological diagnosis is made as follows:

1. Demonstration of the causal organisms from swabs or scraping

These are best taken from early clinical cases and should be placed immediately into transport media. All the bacteria associated with ovine keratoconjunctivitis are readily cultured aerobically on 5 % blood agar. Identification is made according to standard bacteriological techniques. The isolation of *M. conjunctivae* from swabs requires appropriate high-quality artificial media. Under these circumstances, *M. conjunctivae* can be cultured from a high proportion of the samples. The isolation of *Chlamydia* spp. requires embryonated eggs or specially treated cell cultures. Nowadays, polymerase chain reaction (PCR) techniques are becoming available for the detection of *Chlamydia* spp. and offer opportunity for rapid, specific diagnosis.

2. Demonstration of the organisms in tears or conjunctival scrapings

Both *Chlamydia* and *Mycoplasma* species can be detected in smears stained by Giemsa. Interpretation of Giemsa-stained preparations can be confused by the presence of melanin granules. Useful, rapid, and available methods are the indirect or direct immunofluorescence, or techniques adopted from human medicine (Rapid Chlamytest) for direct evidence of *Chlamydia* through a visualised immunological reaction (2).

3. Paired serum samples

Samples of serum should be taken at intervals of fourteen to twenty-one days and examined for rising antibody titres to *Chlamydia psittaci.* Interpretation may be complicated by serological reactions to other chlamydial infections.

Treatment: The most efficient treatment of infectious ovine keratoconjunctivitis is a single intramuscular administration of long-acting formulation of oxytetracycline. Products containing chloramphenicol or penicillin are likely to have an effect on secondary bacterial infections only. A single intramuscular injection of oxytetracycline dihydrate halted further development of clinical conjunctivitis if treatment was given at the onset

of clinical signs. If treatment has been started when the conjunctivitis was at its most severe, a clinical cure can be effected within four days. However, therapeutic treatment has not eliminated *M. conjunctivae* infection. This latent or carrier state may provide a source of infection for uninfected animals and an explanation for the relapse of recovered cases.

Infectious ovine keratoconjunctivitis typically causes more severe clinical signs in adult sheep than lambs. A single intramuscular injection of a long-lasting formulation of oxytetracycline is therefore recommended for the treatment of adult sheep, with the additional application of aureomycin topical powder for severe cases. While parenteral oxytetracycline is also effective for the treatment of sucking or weaned lambs with mycoplasma-associated infectious ovine keratoconjunctivitis, treatment with aureomycin topical powder is generally sufficient. A single application was found to be sufficient in a hill flock, but several daily applications may be required to treat intensively managed lambs.

For lambs affected by *Chlamydia*-associated keratoconjunctivitis, parenteral oxytetracycline is recommended where polyarthritis may be a sequel. Ideally, affected animals should be isolated following treatment, as relapses are common and antibiotic treatment does not eliminate the causal organisms. However, in some circumstances, such as a severe and widespread outbreak, parenteral treatment of all animals with a long-acting formulation of oxytetracycline should be considered in order to suppress the infection.

Prevention: Infectious ovine keratoconjunctivitis occurs sporadically in many flocks, and purchased animals, such as rams, may introduce the disease into previously unaffected flocks. Sheep which are to be introduced to such flocks should be kept isolated for at least two weeks while they are checked for evidence of ocular disease. Though prophylactic treatment with parenteral oxytetracycline may reduce the titre of any infection carried by these animals, it will not eliminate latent *M. conjunctivae* infections.

Control in lambs: The disease is usually so mild that control is not attempted. However, the mixing of affected and unaffected groups is undesirable while clinical disease is present and, subsequently, while latent carriage is likely to be widespread. The provision of adequate space in houses and particularly at feed troughs is also recommended.

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CAUSES OF EMERGENCY SLAUGHTER IN SHEEP AND GOATS AND TRENDS FOR FUTURE DEVELOPMENT

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ABSTRACT

Records from the emergency slaughter of sheep and goats all over the Czech Republic were studied for the period of 1997 to 2002. The causes of emergency slaughter were divided into the following groups: infectious diseases, respiratory diseases, digestive diseases, musculoskeletal diseases, complications *post partum*, diseases of miscellaneous aetiology (disposal of breeding, low efficiency, cachexy, mastitis, injuries, etc.). Trends for future development were determined as an index equal to the ratio of the relative occurrence of the findings during the period of 2000—2002 to the same figures from the period of 1997—1999. A very high occurrence of diseases of miscellaneous aetiology was found (62.65 %) showing a slightly increasing trend (index 1.16), which was found highly significant. Frequent occurrences were also confirmed for musculoskeletal diseases (15.80 %) with an increasing trend (index 1.29) which was also significant. The cases of emergency slaughter were reflected in the total number of condemnations of ovine and caprine carcasses (31.75 %) with a long-term steady trend (index 0.93). Neither a significant increase nor decrease of this value was found. It can be concluded that appropriate measures are necessary for the improvement of the health status of sheep and goats and a reduction in the numbers of emergency slaughter. Such measures should cover both husbandry on farms and handling during transport and should be, in particular, aimed at the prevention of diseases of miscellaneous aetiology and musculoskeletal diseases in sheep and goats.

Key words: condemnation of carcasses; emergency slaughters; findings at slaughterhouses; sheep and goats

INTRODUCTION

Causes of emergency slaughter may serve as indicators of the health status of sheep and goats on farms and during transportation. Reasons for undertaking emergency slaughter indicate the disadvantageous conditions for sheep and goats on the farm and during transportation and may be evaluated in order to propose changes necessary for the improvement of their health status. The causes of emergency slaughter are specified on the basis of findings during the inspection of meat and organs after slaughter. Kofer *et al.* (4) have emphasised the importance of the evaluation of the findings obtained during meat inspection at slaughterhouses. Control activities consist of inspection, palpation, evaluation of cuts in the carcasses and organs of slaughtered animals and laboratory examinations of tissue samples, swabs and impression smears. The aforementioned control activities result in the classification of carcasses as fit for human consumption (edible), fit for processing (conditionally edible) and condemned.

Kozák *et al.* (5) have studied the occurrence of emergency slaughters in selected species of slaughter animals. The authors reported a long-term decrease in the occurrence of condemned ovine carcasses from 6.67 % to 1.64 % and in caprine carcasses from 46.09 % to 6.15 % when comparing the periods of 1989—1994 and 1995—2000.

Kozák *et al.* (6) have studied the occurrence of edible, conditionally edible and condemned carcasses of slaughter animals. The authors have reported a decrease in the occurrence of condemned ovine carcasses in the Czech Republic from 2.11 % to 0.65 % when comparing the periods of 1989—1994 and 1995—2000. The same figures for goats also indicated a decrease, namely from 7.55 % to 2.65 %.

There are very limited literature sources for emergency slaughter in sheep and goats. Studies on slaughtering sheep and goats have focused, for instance, on the comparison of

malformations in lambs detected *post mortem* on farms (2), the monitoring of a single finding, for instance liver fluke in sheep (3), the hygienic evaluation of slaughterhouse technology in sheep (1), the microbiological quality of mutton (7), or the comparison of parameters of carcass quality in goats (8).

The aim of the present work is to determine the most frequent causes of emergency slaughter in sheep and goats and trends in their future development. Furthermore, the impact of emergency slaughter on decisions for the condemnation of ovine and caprine carcasses is also evaluated. A long-term trend in the development of numbers of condemned ovine and caprine carcasses is identified. The results are important for the determination of measures for the improvement of the health status on farms and during the transportation of sheep and goats with regard to emergency slaughter.

MATERIAL AND METHODS

Emergency slaughter of sheep and goats at slaughterhouses during the period of 1997—2002 were studied and the causes of emergency slaughter were evaluated. Veterinary inspectors recorded the findings from emergency slaughter of sheep and goats in the Czech Republic. The findings were divided into the following groups: infectious diseases, respiratory diseases, digestive diseases, musculoskeletal diseases, complications *post partum*, diseases of miscellaneous aetiology (disposal of breeding, low efficiency, cachexy, mastitis, injuries, etc.). Subsequently the decision of the official veterinarian on the classification of carcasses after emergency slaughter was recorded as follows: edible, conditionally edible and condemned.

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

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

RESULTS

Table 1 presents the frequency in absolute figures of the different causes of emergency slaughter in sheep and goats and the results of carcass classification after slaughterhouse inspection. The figures cover individual years from the period of 1997 to 2002.

Table 2 presents the same figures as Table 1 expressed in relative values (in %).

Table 3 presents the causes of emergency slaughters and results of carcass classification after slaughterhouse inspection in absolute and relative figures for the whole period of 1997—2002 and, furthermore, also separately for the periods of 1997—1999 and 2000—2002 in sheep and goats. The trends of their development in the long term expressed as indexes of increase or decrease of relative frequency of different causes of emergency slaughters and results of carcass classification after slaughterhouse inspection are shown in the same Table as well.

It can be concluded from the results that a very high occurrence of diseases of miscellaneous aetiology was found (62.65 %) showing a slightly increasing

Table 1. Breakdown of causes of emergency slaughters in sheep and goats in absolute figures by individual years

Year Causes of emergency slaughter	1997 frequency	1998 frequency	1999 frequency	2000 frequency	2001 frequency	2002 frequency
Infectious diseases	1	4	2	1	0	0
Respiratory diseases	60	26	18	2	8	6
Digestive system diseases	27	19	14	14	12	9
Musculoskeletal diseases	38	25	38	24	45	33
Complications *post partum*	8	8	11	14	6	7
Miscellaneous aetiology	157	140	124	180	122	82
Total	291	222	207	235	193	137
Condemned	86	59	91	67	57	48
Conditionally edible	125	108	74	98	48	28
Edible	80	55	42	70	88	61

Table 2. Breakdown of causes of emergency slaughter in sheep and goats in relative figures by individual years

Year Causes of emergency slaughter	1997 %	1998 %	1999 %	2000 %	2001 %	2002 %
Infectious diseases	0.34	1.80	0.97	0.43	0.00	0.00
Respiratory diseases	20.62	11.71	8.70	0.85	4.15	4.38
Digestive system diseases	9.28	8.56	6.76	5.96	6.22	6.57
Musculoskeletal diseases	13.06	11.26	18.36	10.21	23.32	24.09
Complications *post partum*	2.75	3.60	5.31	5.96	3.11	5.11
Miscellaneous aetiology	53.95	63.06	59.90	76.60	63.21	59.85
Total	100.00	100.00	100.00	100.00	100.00	100.00
Condemned	29.55	26.58	43.96	28.51	29.53	35.04
Conditionally edible	42.96	48.65	35.75	41.70	24.87	20.44
Edible	27.49	24.77	20.29	29.79	45.60	44.53

Table 3. Causes of emergency slaughter in sheep and goats and trends in their development

Causes of emergency slaughter	1997—2002		First period 1997—1999	A	Second period 2000—2002	B	Trend B/A	Signif.
	frequency	%	frequency	%	frequency	%	index	P
Infectious diseases	8	0.62	7	0.97	1	0.18	0.18	0.07
Respiratory diseases	120	9.34	104	14.44	16	2.83	0.20	**0.00
Digestive system diseases	95	7.39	60	8.33	35	6.19	0.74	0.15
Musculoskeletal diseases	203	15.80	101	14.03	102	18.05	1.29	*0.05
Complications *post partum*	54	4.20	27	3.75	27	4.78	1.27	0.36
Miscellaneous aetiology	805	62.65	421	58.47	384	67.96	1.16	**0.00
Total	1285	100.0	720	100.0	565	100.0	1.00	
Condemned	408	31.75	236	32.78	172	30.44	0.93	0.37
Conditionally edible	481	37.43	307	42.64	174	30.80	0.72	**0.00
Edible	396	30.82	177	24.58	219	38.76	1.58	**0.00

Explanations:

* — A significant difference was found between the first and the second period ($P < 0.05$).

** — A highly significant difference was found between the first and the second period ($P < 0.01$).

trend (index 1.16), which was found highly significant ($P = 0.01$). Frequent occurrence was also confirmed for musculoskeletal diseases (15.80 %) with an increasing trend (index 1.29) which was also significant ($P = 0.05$). The cases of emergency slaughter were reflected in the total number of condemnations in ovine and caprine carcasses (31.75 %) with a long-term steady trend (index 0.93). Neither a significant increase nor decrease of this value was found.

DISCUSSION

Sheep and goats are exposed to various loads on farms and during transportation, which may result in the occurrence of various disorders leading to emergency slaughter. The numbers of condemned ovine and caprine carcasses indicate the importance of this load to sheep and goats. On the basis of the determination of the nature of the disadvantageous conditions and of the trends in development based on large numbers of observations during a sufficiently long period it can be determined which measures on sheep and goat farms would lead to improved health status and more advantageous conditions. If the measures are successfully applied, the numbers of condemned ovine and caprine carcasses after emergency slaughter may be reduced.

The frequency of emergency slaughter of sheep and goats showed a decreasing trend, which is in accordance with the results published by Kozák *et al.* (5). We have found that the most frequent causes of emergency slaughter in sheep and goats were diseases of miscellaneous aetiology and musculoskeletal diseases. The frequency of diseases of miscellaneous aetiology

was particularly high. The results cannot be compared with literature sources by other authors, because none have been published specifically for emergency slaughters in sheep and goats. Studies related to slaughtering sheep and goats have been published for instance by Green *et al.* (2), Klimas *et al.* (3), Ellerbroek *et al.* (1), Phil-lips *et al.* (7) and Stankov *et al.* (8). Each study monitored a certain selected factor in this context and no general analysis of causes of emergency slaughters was undertaken.

We have found in our study that the frequency of condemned ovine and caprine carcasses after emergency slaughter is about one third of all cases. There is a steady long-term trend in this parameter. There is however a different trend found for emergency slaughter compared to the data obtained by Kozák et al. (6) from regular slaughterhouses. This can be explained by a general improvement of health status on farms in the long term. Therefore the numbers of condemned ovine and caprine carcasses at regular slaughterhouses has decreased. On the other hand, in cases of individual sheep and goats that suffered from a disease or injury which subsequently led to the decision for emergency slaughter, steps were usually not taken in time and therefore the course of the disease did not allow anything else but the condemnation of the ovine or caprine carcass.

Emergency slaughter of sheep and goats were caused in particular by factors of nutrition, technology of housing, hygiene on farms, postpartum care, and the handling of the animals with regard to the rules of animal protection and welfare on farms as well as during transport and slaughterhouse manipulation. There is a possibility to significantly reduce the frequency of emergency slaughter in sheep and goats through appropriate measures introduced in the areas of animal nutrition, observation of the rules of animal protection and welfare on farms, technology of housing, environment on farms, postpartum care, and handling during transport and at slaughterhouses. The economic impact of emergency slaughter in sheep and goats is currently diminishing but the data from this area necessitates the assessment of the level of nutrition, technology of housing, hygiene and handling of animals. This information source is important not only for farmers but also for the institutions of veterinary administration in charge of inspection on farms, during transport, handling and slaughtering of sheep and goats.

CONCLUSION

The most frequently found causes of emergency slaughter in sheep and goats were diseases of miscellaneous aetiology and musculoskeletal diseases. All causes of emergency slaughter showed a significant impact on the condemnations of ovine and caprine carcasses after emergency slaughter. Overall results demonstrated that the technology of sheep and goat farming produced considerable long-term disadvantages for the animals, which was manifested by diseases of miscellaneous aetiology and musculoskeletal diseases.

Diseases of miscellaneous aetiology and musculoskeletal diseases as causes of emergency slaughters in sheep and goats showed an increasing trend. These groups of diseases have an impact on condemnations of ovine and caprine carcasses after emergency slaughter. As regards the changes, which are necessary for sheep and goat farms in order to improve health status of the animals, it is necessary to implement measures focused on the limitation of diseases of miscellaneous aetiology and musculoskeletal diseases.

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A COMPARISON OF BSDA AND PREMI® TEST SENSITIVITY TO PENICILLIN STANDARDS IN POULTRY MEAT AND AFTER THE ADMINISTRATION OF AMURIL PLV. SOL.

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ABSTRACT

For the screening of antimicrobials in meat and meat products, a new microbiological test, Premi® test has been developed. The sensitivity of the test was evaluated by using the meat juice saturated with penicillin standards and after administration of Amuril plv. sol. The Premi® test sensitivity was compared with the standard *Bacillus stearothermophilus* disc assay (BsDA). Both methods could detect the presence of penicillin's residues below the maximum residue limits (MRL), but detection limits of Premi® test were below the limits of BsDA. The Premi® test is sensitive, reliable, easy-to-use and detects a broad range of antibiotics.

Key words: Amuril plv. sol.; BsDA; penicillin residues; Premi® test

INTRODUCTION

The administration of antimicrobial drugs to food-producing animals may lead to the presence of residues in edible products. Growing concern among consumers and public health authorities about the presence of antibiotic residues in food products requires a better control of both animal production and human food products. As long as antibiotics are used on a large scale in intensive poultry farming, we have to be aware of the risk of their presence in the food chain. Among the concerns regarding the use of antibiotics are antibiotic resistance, potential allergic reactions and technological problems of fermented meat products.

This situation leads to stricter regulations for the occurrence of antibiotic residues in animal food products. Maximum residue limits (0.05 mg.kg^{-1} for penicillin, ampicillin, amoxicillin, 0.3 mg.kg^{-1} for oxacillin, cloxacillin) have been established to prevent too a high concentration of antibiotics. There is the consumer's expectation that the food products do not contain concentrations of antibiotics over the limits.

Therefore the availability of simple and reliable screening systems for the detection of antibiotics is an essential tool in assuring the safety of food products. Recently a new broad spectrum screening test for the detection of antimicrobial residues in meat, Premi® test, has been developed (6, 7).

In our experiment the sensitivity of the Premi® test on different concentrations of five penicillins and Amuril plv. sol. were tested. The results were compared with the standard microbiological BsDA method.

MATERIAL AND METHODS

Penicillins including penicillin, ampicillin, amoxicillin, oxacillin and cloxacillin were purchased from Sigma (St. Louis, MO, USA). Amuril plv. sol. (amoxicillinum ten grams in one hundred grams of powder) was obtained from Lek (Slovenia). *Bacillus stearotermophilus* var. *calidolactis* disc assay (BsDA) consisting of agar and a suspension was obtained from Merck (Germany). The Premi® test was taken from DSM (Netherlands) and Thermoblock (Biotech, Slovakia) was used as a block heater for Premitest ampoule incubation.

Separate stock solutions of twenty-five milligrams per twenty-five millilitres of penicillin, ampicillin, amoxicillin, oxacillin and cloxacillin were prepared by dissolving them in deionised water. Working standard solutions (WSS) and model meat juice samples of tested antibiotics were then prepared as described in Table 1. Meat juice samples for the estimation of the detection limits of tests were extracted by the use of a garlic press from the antibiotic free poultry meat and the meat juice was used as a negative control. WSS were stored in a cool place (two to four degrees Celsius) protected from light.

The *Bacillus stearothermophilus* var. *calidolactis* disc assay (BsDA) utilises a spore suspension of *Bacillus stearothermo-*

Table 1. Concentration ranges of tested antibiotics in model meat juice samples

Antibiotic	Concentration ranges ($mg.kg^{-1}$)
Penicillin G	0.001; 0.002; 0.003; 0.004; 0.005; 0.010; 0.025; 0.050; 0.100
Ampicillin	0.001; 0.002; 0.003; 0.004; 0.005; 0.010; 0.025; 0.050; 0.100
Amoxicillin	0.001; 0.002; 0.003; 0.004; 0.005; 0.010; 0.025; 0.050; 0.100
Oxacillin	0.010; 0.020; 0.030; 0.040; 0.050; 0.100; 0.200
Cloxacillin	0.010; 0.020; 0.030; 0.040; 0.050; 0.100; 0.200

philus var. *calidolactis* inoculated into indicator agar (Merck, Germany). The paper disc soaked with a meat juice standard sample was placed on the surface of the agar. The plates were incubated at 64 °C for three hours and were observed for production of clear zones of inhibition around the sample discs. Inhibition zones are indicated in mm, the positive zone is read at the size above two millimetres.

Premi® test (DSM, The Netherlands) ampoule method for antibiotic residue detection utilises a culture medium containing *Bacillus stearothermophilus* var. *calidolactis*. The Premi® test combines the principle of the agar diffusion test with colour change of the indicator resulting from the active metabolism of the tested microorganism. 100 µl of the meat juice was placed onto the agar in the ampoule and incubated for twenty minutes at room temperature for a prediffusion. The meat juice was not flushed away from the ampoule. The ampoules were incubated for three hours at 64 ± 1 °C and the change of the colour was evaluated.

To determine the sensitivity of the screening tests each concentration was replicated at least four to five times.

In our experiment thirty laying hens (ISA brown 220) were used and the weight average was 2.3 kg. Amuril (dosage 0.5 g/ laying hen/day) was administered with sonda into the oesophagus four times at twenty-four hour intervals. Zero control hens were slaughtered before the beginning of administration and slaughtering continued every day (four hens daily) until the time when the all results were negative.

Meat juice samples were extracted by using of a garlic press from the breast muscles of the laying hens and the meat juice from antibiotic free poultry was used as a negative control.

Data were analysed by Win Episcope 2.0 using test agreement and kappa value for various assay systems comparison.

RESULTS AND DISCUSSION

The results, in which a different concentration of penicillin G, ampicillin, amoxicillin, oxacillin and cloxacillin were added to poultry meat juice are summarised in Table 2. The model meat juice samples were analysed by use of BsDA, and Premi® test.

BsDA produced clear zones of inhibition and the highest sensitive was recorded for penicillin G with minimum detection limit $0.005\ mg.kg^{-1}$. In case of ampicillin and amoxicillin detection limits were 0.010, respectively $0.025\ mg.kg^{-1}$ and the lowest sensitivity was obtained from cloxacillin and oxacillin estimation ($0.040\ mg.kg^{-1}$).

The Premi® test could detect the presence of penicillin G more sensitively than BsDA (kappa > 0.6) with detection limit $0.004\ mg.kg^{-1}$ and similar results were recorded also for ampicillin and amoxicillin ($0.005\ mg.kg^{-1}$) and for cloxacillin and oxacillin ($0.030\ mg.kg^{-1}$).

Both methods, detected the presence of penicillin residues in poultry meat juice below the permitted maximum residue limits established in Food Code ($0.050\ mg.kg^{-1}$) (3).

Bacillus stearothermophilus disc assay (BsDA) is a standard test recommended by Governmental veterinary and food administration (2) for antibiotic residues detection in food and foodstuff of animal origin. As an alternative of BsDA, Premi® test was prepared. This easy-to-use test should guarantee that producers could prevent the risk of penalties because of positive meat samples. The principle of the test is similar to Delvotest SP that is routinely used for antibiotic residue detection in milk in milk industry. The Premi® test should be applied in the same way in meat processing facilities.

Reybroeck (4) has tested concentrations of residues of different substances in the different naturally contaminated meat samples. Each meat sample was obtained from a different chicken and Premitest fulfilled conditions of European legislation. The Premi® test was used by Reybroeck (5) for spiked meat juice samples estimation and the results of Premi® test sensitivity for ampicillin ($0.005\ mg.kg^{-1}$), amoxicillin ($0.005\ mg.kg^{-1}$), oxacillin ($0.025\ mg.kg^{-1}$) and cloxacillin ($0.040\ mg.kg^{-1}$) were comparable with our results (0.005, 0.005, 0.030, $0.030\ mg.kg^{-1}$). Arts and Witkamp (1) have performed improvement of screening of antimicrobial drug residues. Minimum detection levels were $0.0025\ mg.kg^{-1}$ for penicillin G, and $0.005\ mg.kg^{-1}$ for ampicillin and amoxicillin.

Premi® test integrated strategy of antimicrobial compound detection at or below the MRL in a broad spectrum of food products including meat. Proper use of Premi® test will contribute to fewer animals showing positive concentrations of anitbiotics, safer products and better consumer protection.

In our animal study the presence of amoxicillin residues after experimental administration in laying hens was evaluated.

With the BsDA amoxicillin residues were detected in meat juice from the breast muscles, breast and thigh muscles, kidney, heart and liver. A comparison of the zones of inhibition from meat juice samples and muscles placed directly on to an agar medium showed that meat juice produced significantly ($p < 0.005$) larger zones than muscle samples.

Table 2. Results of BsDA and Premi® test means in poultry meat juice sample models

Antibiotics	Tests	Concentration ranges (mg.kg^{-1})						
		0.003	0.004	0.005	0.010	0.025	0.050	0.100
Penicillin G	BsDA*	0.5	0.5	2.0	3.0	3.5	5.0	7.0
	Premi®test	—	+	+	+	+	+	+
Ampicillin	BsDA*	0	0	1.0	2.0	3.0	4.5	6.0
	Premi®test	—	—	+	+	+	+	+
Amoxicillin	BsDA*	0	0	1.0	1.5	2.0	3.5	5.0
	Premi®test	—	—	+	+	+	+	+
		0.010	0.020	0.030	0.040	0.050	0.100	0.200
Cloxacillin	BsDA*	0	0	1.0	2.5	3.5	5.0	7.0
	Premi®test	—	—	+	+	+	+	+
Oxacillin	BsDA*	0	0	1.0	2.0	3.0	4.5	6.0
	Premi®test	—	—	+	+	+	+	+

* — zones of inhibition (mm)

The Premi® test was used for residue determination in meat juice from breast muscles. The Amoxicillin that could be detected with the BsDA could always be detected with Premi® test. The last positive results of BsDA were recorded twenty-four hours after the completion of administration and the Premi® test after forty-eight hours. It means that, in comparison with BsDA, use of the Premi® test in this experiment detection of an amoxicillin residue is possible in poultry muscle for twenty-four hours longer after treatment.

BsDA analysis of heart and kidney showed that all zones of inhibition were below two millimetres and must be considered as a negative. The liver produced positive results only at the time of Amuril plv. sol. administration.

All the results of the BsDA and Premi® tests, on the third day after the completion of drug administration, were negative. On the basis of these results, both methods did not exceed withdrawal period recommended by the producer (two days).

Table 3. Comparison of mean values of BsDA and Premi® test after experimental administration of Amuril plv. sol. in laying hens

Time (hours)	BsDA Breast**	Thigh	Kidney	Heart	Liver	Premi®test (breast muscles)
0*	0/0	0	0	0	0	—
24*	3.3 / 6.7	1.7	1.7	0	2.3	+
48*	4.7 / 7.7	1.7	1.3	1.3	2.0	+
72*	1.8 / 4.7	1.7	1.7	0.7	2.0	+
96	2.3 / 4.0	1.7	0.3	0	1.7	+
120	0.7 / 1.3	0.3	0.3	0	1.0	+
144	0.3 / 0.7	0.3	0.7	0.3	1.3	±
168	0 / 0	0	0	0	0	—

* — Amuril plv. sol. administration

** — Zones of inhibition from breast muscles/breast muscles juice

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THE EFFECT OF CADMIUM AND IRRADIATION ON HISTOLOGICAL AND HISTONE CHANGES IN RAT TESTIS

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ABSTRACT

In this experiment we have studied the influence of cadmium (i. p. 0.5 mg/rat), irradiation (3 Gy) and combination of this effect on histone and histone changes in rat testes. The examination was carried out on the 1st, 7th, 14th and 21th days after irradiation. Cd was applied as a salt $CdCl_2.H_2O$ (Lachema Brno). Before use, the salt was re-suspended in a saline solution of volume 0.5 ml per rat. The rats received 0.5 mg $CdCl_2$/rat 30 min before irradiation. The animals were irradiated with a single whole body dose of 3 Gy by gamma rays from ^{60}Co source (apparatus Chisostat, Czech Republic) with a dose rate of 301 mGy. min^{-1}. We observed that cadmium alone and the combination of cadmium and irradiation caused a deep decrease in the weight and content of histones mainly on the 7th and 14th day. These findings were also confirmed by marked histopathological changes in the testis.

Key words: histones; histopathological changes; irradiation; light microscopy; rat testes

INTRODUCTION

Cadmium is a carcinogenic metal that is nevertheless widely distributed throughout the biosphere and its toxic effects are becoming potentially more serious due to industrialization.

It is known that it is one of the most harmful heavy metals able to induce renal, hepatic and testicular injury (5, 7, 8, 10). Cd is clearly capable of inducing tumors in testes (11,12).

Testes are extremely sensitive and vulnerable to radiation, used as therapy or otherwise, which results in sperm malformation, mutation, chromosomal aberration and DNA and histone loss (1). The vulnerability of different kinds of cell after irradiation has also been described in other organs (2, 9).

In this experimental study we have examined the effect of Cd administration and ionizig irradiation alone and in combination on rat testes.

MATERIAL AND METHODS

Male Wistar rats, chow SPF weighing 200—220 g each, obtained from Velaz (Prague, the Czech Republic) were used in this study. Animals had free access to food and water *ad libitum*, were kept in room 22—24 °C with a photoperiod twelve hours light twelve hours dark and handled with human care. Experiments were performed between 7.00 and 9.00 a. m.

The rats were randomly selected and assigned to four main experimental groups. The four groups of rats were considered as follows:

1. C — non-treated control rats,
2. Cd — application of Cd: 0.5 mg $CdCl_2$/rat,
3. I — irradiation with a single whole body dose of 3 Gy with gamma rays,
4. Cd + I — application of Cd 0.5 mg/rat thirty minutes before irradiation and irradiation with the dose of 3 Gy.

Cd was applied as a salt $CdCl_2.H_2O$ (Lachema Brno). Before use, the salt was re-suspended in saline volume 0.5 ml per rat. The rats received 0.5 mg $CdCl_2$/rat thirty minutes before irradiation..

The animals were irradiatied with a single whole body dose of 3 Gy by gamma rays from ^{60}Co source (apparatus Chisostat, the Czech Republic) with a dose rate of 301 mGy.min^{-1}.

A common histological technique was used to process the testicular tissues for light microscopy. The specimens were fixed in 10 % neutral formol, embedded in parafin and the sections were stained with haematoxilin-eosin.

The isolation of the nuclei was performed according to a modified method of Grunicke *et al.* (3). The testes were cleaned, weighed and cut into the small pieces after excision.

These pieces were homogenized in STKM solution (0.25 mol sucrose, 50 mmol Tris, 25 mmol KCl, 5 mmol $MgCl_2$; pH 7.9) in a glass homogenizer with a teflon piston. The suspension was filtered through cheese cloth and centrifuged for ten minutes at 1 000 × *g*. The pellets were washed in the same solution and then re-suspended in STC buffer (0.34 mol sucrose, 100 mmol Tris, 2 mmol $CaCl_2$) and centrifuged for ten minutes at 1 000 × *g*.

The histones were extracted in 0.2 mol H_2SO_4 (1 h, 0—4 °C). Isolated proteins were precipitated in four volumes of cold 96 % ethanol overnight and centrifuged for fifteen minutes at 4 500 × *g*. The histones were washed with acetone and dissolved in a buffer (0.9 mol acetic acid, 5 mol urea). All procedures were performed at a temperature of 0 to 4 °C.

Protein concentration was determined by the method of Lowry *et al.* (6) using bovine serum albumine as a standard.

Electrophoresis was carried out using the method of Panyim and Chalkley (7). Histones were stained with amido black B. The relative proportions of histone fraction were determined spectrophotometrically on a Shimadzu CS-930 densitometer (Japan).

The experimental data were statistically evaluated by the Peritz' F-test (4) and are given as mean ± S.E.M..

RESULTS

On the 1st day in the group of irradiated (I) rats a rich blood supply was seen in the blood capillaries and the presence of vacuoles in the intestinal tissue were found. In the group of Cd and Cd + I rats haemorraghiae of intestitial tissue and also below the *tunica albuginea* were observed. In some of the tubules seminiferous epithelium lost direct contact with basal lamina. On the 7th day, the group of I rats necrosis and a subsequent depletion of seminiferous epithelium appeared. After administration of Cd degeneration and necrosis of the epithelial cells in the seminiferous tubules were found. A marked increase of intersticial tissue with a number of thickened blood vessel walls was seen as well. In the group of Cd + I rats there were an increased number of cells in the interstitial tissue and necrosis of seminiferous ephitelium.

On the 14th in the group of I rats, the most pronounced changes predominated in the seminiferous tubules at the periphery of the testis. In those tubules interruptions of the basal laminae were found. Marked changes were also seen in the intersticial tissue. The size of Leydig cells was decreased, interstitial space was vacuolated and thickening of the endothelium of blood capillaries apeared. Moreover, *tunica albuginea* showed its thickening and lumen of blood vessels was rich in blood cells. In the groups of Cd and Cd + I rats changes were similar as on the 7th day. On the 21st day in the I group of rats apparent undulance of basal lamina was observed, following depletion of seminiferous epithelial cells. Marked changes were seen in the groups of rats Cd and Cd + I consisting of increased intersticial space and a decreased diameter of the seminiferous tubules. The lumens of those tubules were filled with a necrotic mass.

Administration of $CdCl_2$ to animals caused a marked decrease in the weight of testis on the 7th day and continued to the 21th day. The weight of the testis gradually decreased from the 1st to 21st days after irradiation with the dose of 3 Gy (Fig. 1).

Application of $CdCl_2$ to animals thirty minutes before irradiation with the dose of 3 Gy caused a marked de-

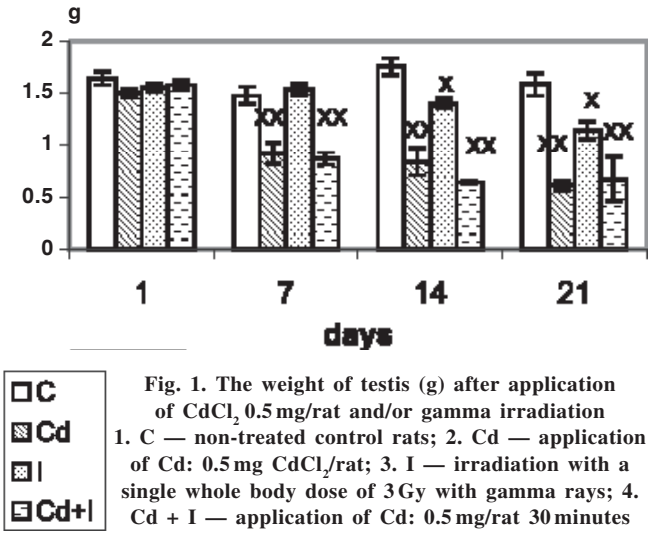

Fig. 1. The weight of testis (g) after application of $CdCl_2$ 0.5 mg/rat and/or gamma irradiation 1. C — non-treated control rats; 2. Cd — application of Cd: 0.5 mg $CdCl_2$/rat; 3. I — irradiation with a single whole body dose of 3 Gy with gamma rays; 4. Cd + I — application of Cd: 0.5 mg/rat 30 minutes before irradiation and irradiation with the dose of 3 Gy

x – P ≤ 0.05, xx – P ≤ 0.01 compared with control group

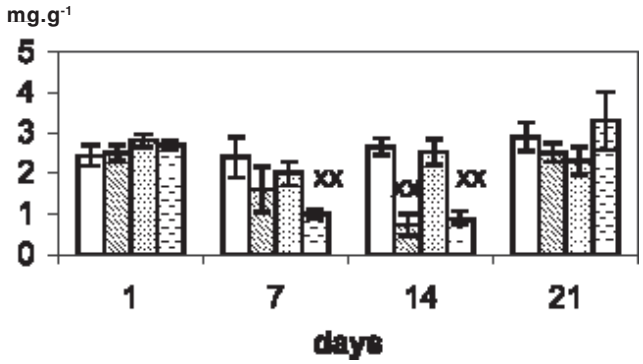

Fig. 2. The concentration of histones in the testis after application of $CdCl_2$ 0.5 mg/rat and/or gamma irradiation For explanation see Fig. 1

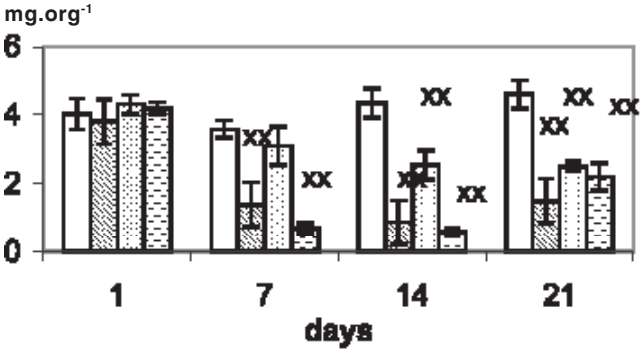

Fig. 3. The content of histones in the testis after application of $CdCl_2$ 0.5 mg/rat and/or gamma irradiation For explanation see Fig. 1

crease in the weigh of the testis. Administration of $CdCl_2$ caused a marked temporary decrease in the content of histone mainly on the 7th and 14th days (Fig. 2). Irradiation with the dose of 3 Gy caused decrease in content. The combination of $CdCl_2$ and irradiation caused on the 7th and 14th days decrease in the content of histones, similar to changes after administration of $CdCl_2$ but the changes were more marked (Fig. 3).

DISCUSSION

The interaction of ionizing radiation or Cd with testes is a complex of events, because the variety of cells differing in radiosensitivity or toxicity. The effects vary with the dosage of radiation or dosage of using Cd.

In our investigation we have studied the sequential effect of cadmium, irradiation and their combination on testicular proteins – histones and histological changes. Gamma irradiation at a dosage of 3 Gy resulted in a gradual decrease in the testicular weight. The weight of the testis was decreased due to loss of germ cells, moreover it was accompained by a decrease in the content of histones. There is a question, if the change of histone content was caused also by inhibition of histone synthesis or by an increase in chromatin condensation and resulting worsened histone extraction.

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MODIFICATION OF POSTRADIATIVE CHANGES OF HISTONES BY BACTERIAL EXTRACT BRONCHO-VAXOM IN RAT TESTES

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ABSTRACT

We have studied the influence of the immunomodulator Broncho-Vaxom (Biogal Pharmaceutical Works, Debrecen, Hungary, under licence from OM Laboratories, Geneva, Switzerland) on the concentration and total content of histones and proportion of individual histone fractions in the testes of rats. Rats were irradiated with a single whole body dose of 6 Gy by gamma rays from ^{60}Co source (apparatus Chisostat, Czech Republic) with a dose rate of 348 mGy/min, twenty-four hours after a Broncho-Vaxom injection. Broncho-Vaxom was administered intraperitoneally at a dose of 1.5 or 3 mg/rat. Animals in all groups were examined on the 1st, 7th and 14th days after irradiation. We have found that administration of Broncho-Vaxom at a dose of 1.5 mg/rat diminished the loss of histones induced by radiation only on the 7th day. Beneficial effect was found also in the relative proportion of histone fractions at a higher dosage of this bacterial extract (3 mg/rat).

Key words: Broncho-Vaxom; gamma radiation; histones; histopathological changes; rat; testes

INTRODUCTION

DNA, in complex with histones and non-histone proteins is arranged in a highly ordered chromatin structure. The core of nucleosomes, the basic subunits of eukaryotic chromatin, is an octamer composed of two molecules of histone fractions H 2A, H 2B, H 3 and H 4 and a stretch of 146 bp of DNA (10, 21, 24). The histone fraction, H1, is associated with linker DNA and links the adjacent nucleosomes. It acts as a general repressor at one level of the regulation of genetic expression by organising nucleosomes into condensed form of chromatin, thereby making the DNA inaccessible to transcription machinery (4, 20, 21).

Ionising irradiation causes profound changes in histones and nucleic acids in the proliferating and quiescent tissue (5, 6, 15, 16). Testes are very sensitive indicators of radiation damage. Changes due to radiation can be prevented by the administration of some radioprotective agents. Radioprotective compounds can be classified as radioprotectants, adaptogens and absorbents.

Immunomodulator Broncho-Vaxom^{R} (BV) is a lyophilized alcalic bacterial extract, which is used as a polyvalent immunotherapeutic agent especially in the treatment of respiratory tract infection (14, 22). It acts also as a stimulator of radioresistance. Therefore BV is included among the adaptogens. Adaptogens are natural protectors, which offer chemical protection against low levels of ionizing radiation. They are generally extracted from the cells of plants and animals and have only minimal toxicity. Adaptogens can influence the regulatory systems of exposed organisms, mobilize the endogenous background of radioresistance and immunity and in this way intensify the overall nonspecific resistance of an organism.

The mechanism of action of BV is not yet fully understood. Experimental studies indicate that it enhances immune responses, both cellular (3, 7) and humoral immune responses (1, 7).

Fedoročko *et al.* (8) have found a radioprotective effect of Broncho-Vaxom on haemopoiesis in mice. The administration of BV twenty-four hours before whole body gamma irradiation accelerated the recovery of haemopoietic stem cells in the bone marrow (CFU-S colony forming units in spleen, GM-CFC-granulocyte-macrophage colony forming cells) and cell numbers in the peripheral blood (19). In the regenerating rat liver, administration of this agent resulted in an alleviation of latent radiation injury, which was indicated by relative increase in the mitotic index, decrease in chromosome aberrations (17) and partially by mitigation of RNA and DNA changes (12).

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

MATERIAL AND METHODS

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

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

The rats were divided into six groups according to the application of tested substance and dose of radiation. Analyses were performed on five to six animals from each group at three time intervals, the 1st, 7th and 14th days after irradiation.

Broncho-Vaxom (Biogal Pharmaceutical Works, Debrecen, Hungary, under licence from OM Laboratories, Geneva, Switzerland) is composed of lyofilized fractions of the eight most common bacteria of the upper respiratory tract (*Haemophilus influenzae, Diplococcus pneumoniae, Klebsiella pneumoniae, Klebsiella ozaenae, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans, Neisseria catarrhalis).* The preparation is free of endotoxins (less than 0.0002 % by the *Limulus* and pyrogenicity test) (2). Just before use, the drug was resuspended in PBS (pH 7.4) in a volume 1.2 ml (1.5 mg or 3 mg) per rat and administered intraperitoneally. Control animals received 1.2 ml PBS at the same time.

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

1. C — non-treated control rats
2. I — irradiation with the dose of 6 Gy
3. and 4. BV 1.5, BV 3 — application of Broncho-Vaxom (1.5 or 3 mg/rat)
5. and 6. BV 1.5 + I, BV 3 + I — application of Broncho-Vaxom (1.5 or 3 mg/rat) twenty-four hours before irradiation with the dose of 6 Gy.

Isolation of nuclei and histone extraction was carried out according to the method of Grünicke *et al.* (11).

Protein concentration was determined spectrophotometrically (Hitachi, Tokyo, Japan) by the method of Lowry *et al. (18)* using bovine serum albumin as a standard.

Electrophoresis was carried out using the method of Panyim and Chalkley (23).

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

Experimental data were statistically evaluated by Peritz' F-test (13). They are given as mean S.E.M. on the figures and tables.

RESULTS

Administration of Broncho-Vaxom did evoke significant changes on the 1st day after the dose of three milligrams per rat both in the concentration and content of histone, to compare with the control value (Fig. 1A, B).

Irradiation with the dose of 6 Gy caused a deep temporary decrease in concentration of histones on the 1st day which was followed by increase on the 14th day after irradiation. Because of a concomitant increase in testis weight, the decrease in concentration was not accompanied by a decrease in the total content of histones. The total content of histones decreased only on the 1st and 7th days after irradiation with the dose of 6 Gy.

Administration of Broncho-Vaxom to animals irradiated with the dose of 6 Gy alleviated the decrease in histone concentration and content in the dose of 1.5 mg/rat only on the 7th day. At earlier intervals of investigation, however the decrease in histone concentration and content in

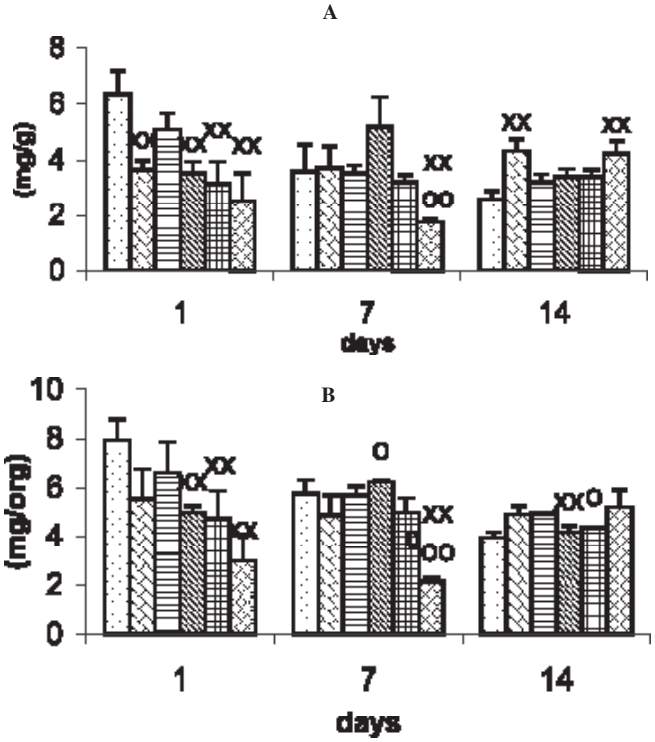

Fig. 1A + 1B: The concentration (A) and content (B) of histones in the testes of rats after application of Broncho-Vaxom (1.5 or 3 mg/rat) and/or gamma radiation 6 Gy

1. C — non-treated control rats
2. I — irradiation with the dose of 6 Gy
3. and 4. BV 1.5, BV 3 — application of Broncho-Vaxom (1.5 or 3 mg/rat)
5. and 6. BV 1.5 + I, BV 3 + I — application of Broncho-Vaxom (1.5 or 3 mg/rat) 24 h before irradiation with the dose of 6 Gy

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

Table 1. The relative proportion of histone fractions (%) in the normal liver of rats after application of Broncho-Vaxom (1.5 or 3 mg/rat) and/or gamma radiation 6 Gy

Groups	1st day			7th day			14th days		
	H1	H2+ H3	H4	H1	H2+H3	H4	H1	H2+H3	H4
C	**19.2 ±1.5**	**67.7 ±1.1**	**13.1 ±0.8**	**14.95 ±1.19**	**74.15 ±3.28**	**21.25 ±0.65**	**21.51 ±0.84**	**62.13 ±2.35**	**18.26 ±2.32**
I	9.91^{xx} ±0.65	75.61^{xx} ±1.16	15.59 ±2.06	15.81 ±1.25	70.28 ±1.66	16.04^{xx} ±0.78	18.55 ±1.26	61.97 ±4.07	18.57 ±3.74
BV 1.5	11.62^{xx} ±0.72	66.69 ±1.56	19.57^{xx} ±1.10	15.17 ±0.35	67.4^{x} ±1.7	21.55 ±2.04	22.21 ±1.61	62.31 ±2.71	20.36 ±2.54
BV 1.5 + I	12.64^{xx} ±0.11	72.73^{xx} ±1.02	15.58 ±0.93	14.74 ±2.72	65.33^{xx} ±1.56	22.08^{oo} ±1.22	18.99 ±0.45	61.49 ±0.61	20.49 ±0.42
BV 3	11.21^{xx} ±1.06	70.96 ±1.16	21.27^{xx} ±1.29	14.08 ±2.83	69.35 ±5.26	20.0 ±0.54	17.3^{x} ±0.53	62.98 ±0.59	20.98 ±0.12
BV 3 + I	18.32^{oo} ±1.63	67.58 ^{oo} ±4.02	12.97 ±3.17	10.54^{xo} ±0.17	65.67^{xx} ±0.29	24.61^{xoo} ±0.37	14.49^{xx} ±0.9^{oo}	68.05^{xx} ±1.22^{oo}	20.40 ±1.79

Legend:

1. C — non-treated control rats
2. I — irradiation with the dose of 6 Gy
3. and 4. BV 1.5, BV 3 — application of Broncho-Vaxom (1.5 or 3 mg/rat)
5. and 6. BV 1.5 + I, BV 3 + I — application of Broncho-Vaxom (1.5 or 3 mg/rat) 24 h before irradiation with the dose of 6 Gy

x — P≤0.05, xx — P≤0.01 compared with control group

o — P≤0.05, oo — P≤0.01 compared with the corresponding non-protected irradiated groups

rats pretreated with the higher dose of Broncho-Vaxom was even deeper in comparison with the non-protected irradiated animals.

As it is shown in the Table 1, there were differences observed between the groups of animals treated with Broncho-Vaxom alone besides a decrease in relative proportion of fraction H 1 and concomitant increase in fraction H 4 on the 1st day of examination.after a dose of 1.5 or 3 mg/rat.

The total body irradiation of rats with a dose of 6 Gy caused a deep decrease in the fraction H1 accompained by an increase in the H 2 + H 3 fractions on the 1st day. On the subsequent days the relative proportion of these fractions was restored. In the fraction H 4 a transient decrease was found on the 7th day after irradiation.

Application of Broncho-Vaxom twenty-four hours before irradiation alleviated most of the histone fraction changes mainly at a dose of 3 mg/rat. On the 14th day after combined treatment 6 Gy + 3 mg Broncho-Vaxom per rat, however, changes in the histone fraction proportion were even higher than after irradiation alone.

DISCUSSION

In previous experiments, Fedoročko *et al.* (8, 9) have found an increased survival of mice irradiated with a lethal dose of gamma irradiation and protected by the administration of Broncho-Vaxom twenty-four hours before irradiation. Administration of BV 24 h before irradiation with lower doses of gamma radiation had a beneficial effect on haemopoiesis (8, 9) and on the radiation-induced changes of nucleic acids in rat tissues differing in respect of proliferative activity and radioresistance. The beneficial effect of the immunomodulator on the development and recovery of nucleic acids changes was demonstrated in haemopoietic tissues of bone marrow and spleen (12). In the testes the effect of Broncho-Vaxom was less noticeable in the dose of 1.5 mg/rat but more in the dose of 3 mg/rat.

Broncho-Vaxom induces secretion of cytokines (IL-1 and prostaglandins), the radioprotective effect of which is known under both *in vitro* and *in vivo* conditions (25) suggesting a possible mechanism of the radioprotective effect of this preparation, which was confirmed in the proliferating tissues of the bone marrow and spleen in nucleic acids, in our experiment in the testes at the level of histones.

Histone H 1 is not present in the nucleosome core, but it joins neighbouring ones. Histone H 1 is more sensitive to gamma irradiation because it is not protected within the nucleosome core. The decrease in the relative proportion of histone fraction H 1 was probably connected with its poor extraction accompanying a drop in

transcriptional activity. We conclude that a decrease in the histone concentration and total content of testicular histones was caused mainly by a drop in the histone fraction very rich in lysin.

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CHANGES IN NEUTROPHIL FUNCTIONS AFTER SIMPLE SURGERY

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ABSTRACT

The effect of surgery on the phagocytic activity of blood neutrophils, iodnitrotetrazolium (INT) reduction and chemotaxis of polymorphonuclear cells was studied in sixteen dogs. Simple ovariohysterectomy with anesthesia induced by ketamine and xylazine; and ketamine, xylazine and halothane caused short depression of phagocytic activity that persisted for four hours after surgery. Ingestion capacity of leukocytes decreased significantly immediately and four hours after surgery. More conspicuous and longer decrease of INT activity and chemotaxis of phagocytes was found after anesthesia with ketamine and xylazine in the lower dose and maintained with halothane than after the use of ketamine and xylazine in a dose maintaining anesthesia. However, these differences between the two anesthetic techniques were not significant.

Key words: anesthesia; chemotaxis; dogs; INT; ovariohysterectomy; phagocytosis

INTRODUCTION

Neutrophils play an important role in the antibacterial host defense mechanism and in the pathogenesis of tissue injury. Pain, stress, necrotic tissue and invading microorganisms are known modulators of the immune response of patients undergoing surgery. Anesthesia combined with surgery may alter the immune function with potential impact on the postoperative course (1). Impaired immunity, often observed after surgery, is multifactorial (18). Surgical stress and anesthetic agents can induce an alteration in various aspects of polymorphonuclear leukocyte functions. In case of their dysfunction, an increase of susceptibility to infection occurs (13).

In human populations postoperative immunosuppression is well documented (15, 22). Various anesthetics (propofol, thiopentone, midazolam, ketamine) are known to depress human neutrophil functions (3, 12, 14, 20). The significant reduction of cellular immunity by surgical stress in human patients has also been described (6, 19).

There are only limited data concerning immunosuppression associated with anesthesia and surgery in animals. Lymphocyte blastogenesis depression has been described after simple laparotomies (8) and ovariohysterectomy (11) in dogs.

The aim of the present study was to observe the effect of the surgical procedure using two anesthetic techniques on phagocytosis, the ingestion ability of neutrophils, chemotaxies and iodnitrotetrazolium reduction by polymorphonuclear cells in dogs.

MATERIAL AND METHODS

Animals: Sixteen healthy female dogs with an average age of 4.2 years of different breeds undergoing ovariohysterectomy. For premedication diazepam and atropine i.m. were used. In eight of the dogs (group A) anesthesia was induced and maintained with intramuscular application of xylazine (2 mg. kg^{-1}) and ketamine (10 mg.kg^{-1}). In eight of the dogs (group B) ketamine and xylazine were used in a low dose (one third of the dosis commonly used) intravenously and anesthesia was maintained with mixture of halothane, nitrous oxide and oxygen. The duration of anesthesia ranged from two to three hours and the duration of surgery from one to two hours. There were no major operative and postoperative complications in any dog.

Control group (C): six healthy female dogs with an average age of 3.1 years of different breeds served as control animals.

Blood collection

Peripheral blood samples were obtained by *v. cephalica* puncture and placed into a tube containing heparin. Blood collection was carried out according to the following scheme:

1. before anesthesia; 2. immediately after surgery; 3. four hours after surgery; 4. twenty-four hours after surgery; 5. forty-eight hours after surgery; 6. seven days after surgery.

The evaluation of parameters of cellular immunity

The phagocytic activity of blood neutrophils was examined as described by Větvička *et al.* (23). 0.1 ml of fresh heparinized blood (5 U of heparin in one ml of blood) was mixed with 0.05 ml of 2-hydroxyethylmetacrylate particles (MSHP, diameter 1.2 mm; ARTIM Prague) and incubated for one hour at 37 °C with occasional shaking. The phagocytic activity (PA) of neutrophils (Ne) was expressed as the percentage of the cells phagocytizing three and more MSHP and as the index of phagocytic activity (IPA) – representing the ingestion ability of neutrophils (the ratio of the number of phagocytized MSHP and the number of all potentially phagocytizing Ne).

The metabolic activity of phagocytes was evaluated using the iodnitrotetrazolium reduction test (INT) in modification according to Mareček and Procházková (10). The index of metabolic activity (IMA) was calculated from the metabolic activity of cells stimulated by zymozan and the metabolic activity of unstimulated phagocytes.

The chemotactic activity of polymorphonuclear cells was determined using the test of chemotaxis under agarosis in a modified version (10). Suspension of isolated polymorphonuclear cells (PMNL) was added into a well in agarose. Rabbit serum activated with zymozan was used as a chemotactic factor. The chemotaxis of PMNL was evaluated from a spontaneous and evoked migration line after incubation and expressed as a chemotactic index.

Statistical analyses. The immunological parameters of surgical patients with different anesthesia were compared between them and with those in healthy dogs and analyzed with the Mann-Whitney U test.

RESULTS

As shown in Fig. 1 the percentage of the phagocytizing neutrophils in groups A and B significantly decreased immediately and four hours after the end of surgery. Twenty-four hours after surgery this parameter was still lower in both groups of surgical patients comparing to presurgical values as well as control group. Later, the phagocytic activity of neutrophils in groups of dogs after ovariohysterectomy gradually reached the values of the control animals and was comparable with presurgical values. No significant differences were present comparing the two groups of dogs undergoing ovariohysterectomy.

The ingestion capacity of neutrophils expressed as a phagocytic index (Fig. 2) decreased in both groups of surgical patients significantly immediately after surgery, insignificantly four hours after surgery. Then, a gradual increase to the values comparable with healthy dogs was observed. No difference was present in a comparison of groups A and B.

The metabolic activity (MA) of phagocytes evaluated by INT test and expressed as an index of MA (Fig. 3), was reduced after surgery in both groups of surgical patients in comparison with control values. In *group A*, this decrease was significant only in sample 2 (immediately after surgery), in subsequent samples there were no significant differences compared with the control. In *group B*, the decrease of the index of metabolic activity was not significantly lower immediately after surgery, but later on in samples 3, 4, 5 there was a significant suppression of this parameter. Although the differences between groups of patients treated with two kinds of anesthetic techniques were not significant, the reduction of metabolic activity of phagocytes was more pronounced and of longer duration in *group B* (anesthesia maintained by halothane).

The chemotaxis of polymorphonuclear cells was changed in *group A and B* insignificantly as shown in Fig. 4. A slight suppression of chemotactic activity was found in sample 3 (four hours after surgery) in *group A,* in all subsequent samples this parameter gradually increased. In *group B* chemotaxis was gradually reduced in sample 2 until sample 5, but without statistical significance.

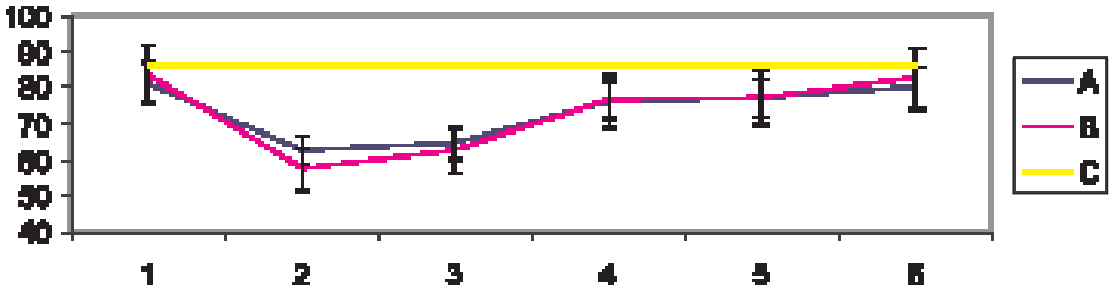

Fig. 1. Phagocytic activity of neutrophils

Legends: Sample 1 (pre-surgery), 2 (immediately after surgery), 3 (4 hours after surgery), 4 (24 hours after surgery), 5 (48 hours after surgery), 6 (7 days after surgery)

Group A*: significant differences ($p < 0.05$) in samples 2, 3 *versus* pre-surgical value and *group C
Group B*: significant differences ($p < 0.05$) in samples 2, 3 *versus* pre-surgical value and *group C

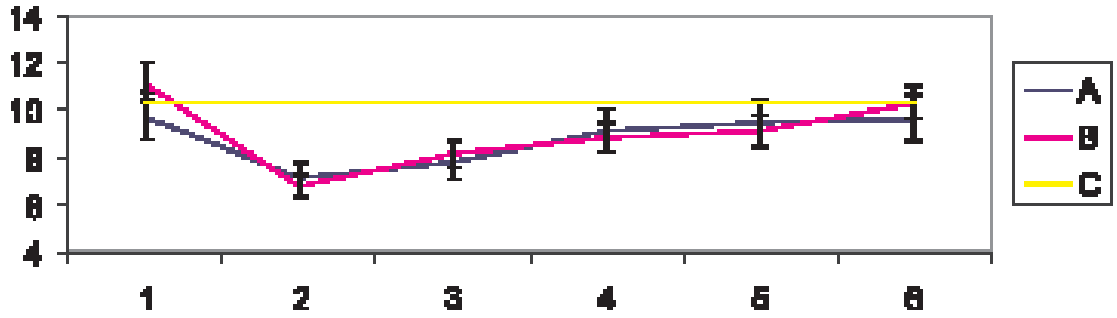

Fig. 2. Phagocytic index of neutrophils

Legend for samples see Fig. 1

Group A*: significant differences ($p < 0.05$) in samples 2 *versus* pre-surgical value and *group C
Group B*: significant differences ($p < 0.05$) in samples 2, *versus* pre-surgical value and *group C

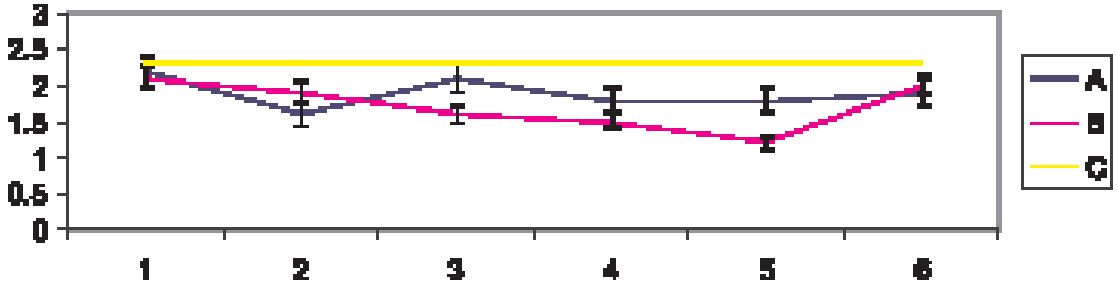

Fig. 3. Index of metabolic activity of phagocytes

Legend for samples see Fig. 1

***Group A*: significant differences ($p < 0.05$) in sample 2 *versus* pre-surgical value and group C**
Group B:* significant differences ($p < 0.05$) on samples 3, 4, 5 *versus* pre-surgical value and *group C

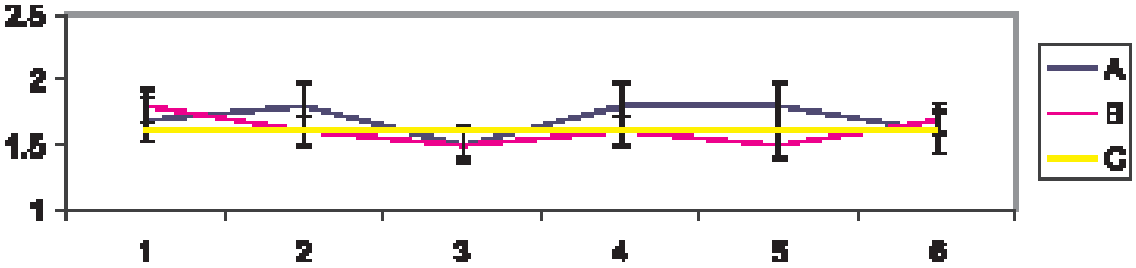

Fig. 4. Chemotactic activity of PMNL

Legend for samples see Fig. 1

Group A: no significant differences *versus* pre-surgical value and group C
Group B: no significant differences *versus* pre-surgical value and group C

When comparing the two groups of surgical patients, a more severe and longer suppression was found out in patients from *group B*. Yet this group difference was also not significant.

DISCUSSION

Surgical stress accompanied by trauma and pain in combination with the effects of various anesthetics may cause an alteration of immune function with an impact on the postoperative course. Initial resistance of bacterial infection is mediated primarily by polymorphonuclear cells. Anesthetic agents have been reported to impair PMNL function *in vitro* condition (12). A depressed host defense mechanism during the recovery period is a limiting factor for the risk of secondary complications.

In the dogs under our study, the marked reduction of phagocytic ability of neutrophils that persisted twenty-four hours after surgery was found. Metabolic activity of phagocytes was also altered in surgical patients immediately after surgery; even in the group of dogs where anesthesia was maintained with xylazine this parameter was suppressed for forty-eight hours. A similar course

of the values of chemotactic activity was found with a longer depression of this function in dogs after combined anesthesis with ketamine, xylazine and halothane with nitrous oxide.

Phagocytosis provides one of the body's first-line defenses against invading bacteria. Alteration of any function of phagocytes can result in postoperative infection. Phagocytosis is known to be inhibited by various anesthetics. Nishina et al. (14) found that thiopental, midazolam and ketamine impaired chemotaxis, phagocytosis and oxygen radicals production. However, no significant depression of phagocytosis and chemotaxis after the use of clinically relevant concentrations of clonidine and dexmedetonine was described (15).

Results from Davidson *et al.* (3) suggest that other anesthetic agents (propofol, thiopentone, midazolam and ketamine) have minimal effects on phagocytosis; only higher concentrations of thiopentone and ketamine may affect phagocytic function. Also halothane has little effect on the chemotactic activity of human neutrophils (5). Latex particle phagocytosis and reduction of nitroblue tetrazolium was inhibited during general anesthesia in patients anesthetized with halothane and nitrous oxide (2) that is in agreement with our study. Similarly, Heine *et al.* (7) have confirmed a propofol dose-dependent inhibition of phagocytosis and superoxid anion production during the respiratory burst of PMNL. On the other hand Nunn *et al.* (16) have reported that halothane does not inhibit human neutrophil function.

Postoperative immunosuppression is not so well documented in animals. Lewis *et al.* (9) have demonstrated halothane-induced suppression of cell-mediated immunity in tumor-bearing mice. The natural course of infectious diseases in mice was significantly modified by anesthetic-induced immunosuppression (4). Study of immunosuppressive effect of ovariohysterectomy and laparotomy in dogs has shown various degrees of immune function alteration (8, 11, 21) considering lymphocyte functions. There are only limited data concerning suppression of the neutrophil functions in animals after surgery. A significant decrease of phagocytic activity of cows undergoing surgical excision of skin papillomas after use of xylazine has been found in a study by Paulík *et al.* (17). These findings indicate that besides surgery alone also type and dose of anesthetic may influence post surgical immunosuppression.

On the basis of present study we can conclude that significant alteration of the function of phagocytes after simple ovariohysterectomy was of short duration and although no significant differences were found comparing patients anesthetized with two kinds of anesthetic techniques (ketamine xylazine and ketamine, xylazine and halothane), suppression of phagocyte function persisted for a longer time in dogs maintained in anesthesia with xylazine and halothane.

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THE INFLUENCE OF A MINERAL-HERBAL MIXTURE ON THE MINERAL ELEMENT LEVEL IN KID GOAT SERUM

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ABSTRACT

Mineral deficiency in the trophic system soil-plant-animal was investigated in the south-eastern part of Poland where a goat farm of a white improved breed was situated. The deficit concerned the elements Mg, Fe, Zn, and Cu. Kid goats were fed a mineral-herbal mixture appropriate for the region of animal habitation. The studies of kid goat serum showed a favourable impact of the mixture which increased the levels of deficit elements as well as calcium, phosphorus and sodium.

Key words: goatlings; mineral elements; serum

INTRODUCTION

Imbalance in mineral nutrition is of a variable nature. In ruminants it results most frequently from supplying feeds produced in their respective region. Preliminary examination conducted in kid goats from the south-eastern Poland showed that their serum mineral element levels varied considerably. This created grounds for the studies on mineral nutrition of kid goats fed a new mineral-herbal mixture, prepared according to the authors' formula, with the aim of compensating for the mineral deficiency of important elements. The effect of the mixture was investigated by means of the determination of the serum levels of Ca, P, Mg, Na, K, Fe, Cu, and Zn.

MATERIALS AND METHODS

Studies were carried out on a farm "Z" in the Lublin province over one year. Out of a goat herd of improved white breed two groups (control and experimental), fifteen kid goats in each, were formed for further examination. The animals were of similar habit, physical condition and age. They were kept under the same micro-climatic and environmental conditions.

In the first stage of life the kids received only mother's milk. They were weaned at the turn of April/May, at the age of five weeks and in the subsequent stage they received the same rations as adult goats. Serum samples were taken at the age of five, eight and twelve weeks (Table 3). They were maintained in an alcove-pasture system. The feeding was based only on feeds produced on the farm according to the *Feeding Standards of Farm Animals* (3). During the autumn-winter season their rations consisted of meadow hay, barley straw, dry marc, fodder beet and mixture rich in cereals, while in summer the animals grazed on pasture. The rations were supplemented with hay and fodder straw.

Feed rations of the experimental group were supplemented with a mineral-herbal mixture developed by the Laboratory of Reproduction Biology, UA in Lublin. The kids were supplied fifty grams daily of the mixture; it consisted of dolomite — 55 %, phosphate (from Bonarka) — 10 %, fodder chalk — 3 %, NaCl — 5 %, flax — 5 %, chamomile — 4 %, nettle — 8 %, willow bark — 4 %, horsetail, mellilot, and oak bark — 6 %. In order to determine the serum content of the elements examined, three samples of blood were withdrawn from the kids at five, eight and twelve weeks of age from the *superficial jugular* vein by a clotting method in the early hours prior to feeding.

The serum was examined quantitatively for the following elements: Ca, Mg, Na, K, Fe, Cu, and Zn. We employed an atomic absorption spectrophotometry method using a ASA-Unicam 939 apparatus. Inorganic phosphorus was determined by the method of Fiske-Subbarow (9).

Two soil samples were obtained from the pastures and arable land with a sampling rod from the depth of nought to fifteen centimetres at the height of the vegetative season (in June and September).

Table 1. Means ($\bar{x}$) and standard deviations (SD) of macro- and micro-elements in feeds (n = 40)

Examined feed		Ca	P	Mg	Na	K	Fe	Cu	Zn	Mn	Co
		g.kg^{-1} d.m.					mg.kg^{-1} d.m.				
Pasture fodder	$\bar{x}$	8.5	3.0	1.0	1.3	16.4	120.0	3.6	37.8	35.4	0.
	SD (±)	3.8	2.0	0.6	0.2	4.0	18.0	1.2	1.0	5.0	0.1
Meadow hay	$\bar{x}$	7.3	2.9	1.3	1.8	18.2	120.8	3.2	32.0	40.2	0.5
	SD (±)	2.8	1.8	0.6	0.2	3.0	17.0	1.0	11.0	5.0	0.2
Wheat bran	$\bar{x}$	9.8	7.0	4.6	54	17.7	132.4	9.7	80.2	39.0	0.5
	SD (±)	1.7	0.9	1.6	1.2	3.9	11.0	1.4	18.0	4.8	0.1
Oats straw	$\bar{x}$	2.2	0.6	0.4	1.6	15.0	115.0	0.4	20.7	21.5	0.1
	SD (±)	0.5	0.1	0.1	0.4	3.0	8.6	0.1	4.1	1.4	0.2
Oats meal	$\bar{x}$	14.8	8.8	4.3	7.0	19.0	140.0	9.8	80.8	38.0	0.3
	SD (±)	5.0	1.4	1.4	1.3	3.8	12.0	1.5	18.1	5.0	0.2
Barley meal	$\bar{x}$	13.8	8.8	4.6	8.1	18.9	142.1	10.8	81.1	37.0	0.2
	SD (±)	4.3	1.5	1.5	1.4	3.6	9.9	1.3	18.0	4.1	0.1
Dry marc	$\bar{x}$	13.2	1.0	0.9	4.4	10.4	144.5	0.9	35.6	27.4	0.09
	SD (±)	0.5	0.05	0.02	0.3	0.03	1.2	0.2	0.5	0.5	0.01

$\bar{x}$ — mean values; SD — standard deviation

Representative samples of feedstuffs were examined regularly as they were used to form the rations. The content of Ca, Na, Mg, K, Fe, Cu, Zn, Co, and Mn was determined in the soil and feeds analogically to that in the serum. The supply of minerals to goats determined according to the dietary units and analysis of feed mineral composition. Two-directional analysis of variance was performed and the significance of the interactions between the sampling dates and concentration values of particular elements was determined by Fisher's test. Any significant influences observed for the respective sampling dates were analysed in detail by detailed comparisons of the means with Tukey's test.

Table 2. Mean daily supply of minerals to goats

Element	Unit	Feeding type summer	winter
Ca	g	15.9	20.4
P	g	7.1	9.9
Mg	g	3.3	5.8
Na	g	5.1	8.4
K	g	39.8	61.3
Fe	mg	265.0	395.0
Cu	mg	8.9	9.91
Zn	mg	115.8	107.3
Co	mg	0.9	1.0
Mn	mg	93.3	119.2

RESULTS AND DISCUSSION

The farm was situated on loessial soil with a high proportion of sand. The soil had a slightly acidic reaction (pH = 6.1) and contained from 1.5 to 2 % of humus. The soil in the goat-keeping region had high level of calcium (17.3 ± 2.2 g.kg^{-1} d. m.) and zinc (196.0 ± 30.0 mg. kg^{-1} d. m.) and average content of Fe (9.9 ± 1.5 mg.kg^{-1} d. m.) and Mn (226.0 ± 30.0 mg.kg^{-1} d. m.). It was poor in P (2.8 ± 0.4 g.kg^{-1}), Mg (1.8 ± 0.2 g.kg^{-1} d. m.), and Cu (4.4 ± 0.3 mg.kg^{-1} d. m.) (6).

Optimum levels of elements per kg of goat feed are as follows: 3.0 g P, 1.5—2.0 Mg, 30—60 mg Fe, 75 mg Zn, and 5—10 mg Cu. The content of P, Mg, Cu, Zn, and Mn in feeds was low in the majority of samples (Tab. 1). As to the other elements, their level in feed samples reached average values (2).

The content of minerals in feedstuffs (Table 2) shows substantial differences in mineral supply of goats in individual feeding stages. The feeds under study failed to supply goats with a sufficient amount of minerals, particularly with regard to P, Mg, Cu and to some extent also Zn (5).

Table 3 compares the means of serum levels between the kid goat groups and shows statistically significant differences for Ca, P, Mg, and Cu in favour of group D. No significant differences were observed for K, Fe, and Zn. Characteristic was a low level of Cu in the serum of kids, particularly at the age of twelve weeks.

Comparison of the means between the sampling periods showed a decreasing tendency for the elements. Statistically significant differences were recorded for Ca, P, Na, and Fe between the first sampling date and the others, while those for Cu and Zn were significant for all the sampling dates. Metabolism of microelements including Cu depends to a considerable degree on genetic factors that underlie the interspecies and interbreeding

Table 3. Mean values ($\bar{x}$) and standard deviations (SD) of Ca, P, Mg, Na, K [mmol.l^{-1}], Fe, Cu, Zn [µmol.l^{-1}] in kid goat serum

Element	Sampling date												Means for groups		Means for dates		
	5th week of life				8th week of life				12th week of life								
	$\bar{x}$	(±) SD	$\bar{x}$	(±) SD	$\bar{x}$	(±) SD	$\bar{x}$	(±) SD	$\bar{x}$	(±) SD	$\bar{x}$	(±) SD	$\bar{x}$ C	$\bar{x}$ D	$\bar{x}$ T1	$\bar{x}$ T2	$\bar{x}$ T3
Ca	2.7	0.11	2.9	0.15	2.4	0.12	2.7	0.21	2.4	0.11	2.6	0.12	2.5^{a}	2.7^{b}	2.8^{b}	2.5^{a}	2.5^{a}
P	1.2	0.22	1.4	0.11	1.0	0.18	1.1	0.13	0.9	0.16	1.2	0.22	1.1^{a}	1.2^{b}	1.3^{a}	1.1^{b}	1.1^{b}
Mg	0.9	0.15	1.2	0.12	0.8	0.15	1.0	0.12	1.0	0.19	1.1	0.31	0.9^{a}	1.1^{b}	1.0^{b}	0.9^{a}	1.1^{b}
Na	140.2^{ax}	4.92	145.9^{by}	5.89	137.8^{ax}	2.55	138.5^{ax}	3.0	137.9^{ax}	2.20	139.5^{ax}	3.1	138.6^{a}	141.5^{b}	143.3^{b}	138.0^{a}	138.8^{a}
K	4.6^{by}	0.29	4.2^{ex}	0.39	4.1^{ax}	0.33	4.3^{ax}	0.21	4.2^{ax}	0.38	4.4^{ex}	0.4	4.3	4.3	4.4	4.2	4.3
Fe	22.4^{bx}	1.39	23.9^{by}	1.48	22.8^{by}	1.12	21.4^{ex}	1.22	20.8^{ax}	1.51	22.01^{ey}	0.9	22.0	22.4	23.1^{b}	22.1^{a}	21.4^{a}
Cu	10.9^{cx}	1.51	13.7^{gy}	1.44	10.0^{bx}	0.85	11.3^{fy}	0.6	8.8^{ax}	0.6	9.7^{ey}	0.8	9.9^{a}	11.6^{b}	12.3^{c}	10.8^{b}	9.2^{a}
Zn	23.4	0.97	23.8	0.68	22.0	1.43	21.5	1.51	20.9	1.25	20.9	1.6	22.1	19.8	23.6^{c}	21.8^{b}	20.9^{a}

x — arithmetic means; SD — standard deviation; C — control group; D — treatment group; significance of differences between the groups were marked with upper case letters; the means differ significantly ($P<0.05$) if not marked with the same letter

a, b, c, d — statistically significant differences between mean values for the sampling dates at control for interaction groups x dates and between the means within the groups and dates;
e, f, g, i — statistically significant differences between mean values for sampling dates at control for interaction groups x dates;
x, y — statistically significant differences between mean values of the control and the treatment group at sampling date.

differences between individual animals. This may relate to absorption, transport, storage, elimination, binding through receptors or intracellular conversion. The differences in the supply of Cu and Co may frequently overshadow the adaptation abilities, particularly of young animals. Moreover, the content of mineral elements in the serum and hair of animals is subject to variations in the first stage of their life. It can be the consequence of the increasing body weight of the kids or variations of mineral elements in their mother's milk. One can speculate that as with sheep, the mechanism of uptake of Cu is related to the balance between elements and proteins and amino acids that acts as ligands mediating the transfer of Cu to cells of the liver.

The mineral mixture was supplemented with a herbal additive to improve its palatability and some technological characteristics (prevention of fractionation) and to increase the availability of macro- and microelements. It has been assumed that combination of mineral components and herbs stimulates, due to the presence of biologically active substances, secretion of digestive juices in the alimentary tract, enhances the passage of gastric contents and improves the availability of minerals (1, 8).

The high levels of Ca limit the availability of P, Mg, Cu, and Zn due to the natural antagonism between these elements. Low pH values result in a decreased availability of many elements, e.g. copper (6). The content of zinc in the soil is conditional upon both the type of parental rock and atmospheric pollution. Acidic soils release this element ten times faster than alkaline soils, the more so because zinc occurs in the soil in an ionic form and the stability of its organic complexes is generally low (7, 8).

We should mention here once more the specificity of goat feeding as these animals consume unconventional feedstuffs like weeds, herbs, sprouts of trees and shrubs. If cows, sheep and goats graze the same pasture lots cows will consume 17, sheep 20 and goats 90 different plants. This results in an additional and differentiated mineral supply of these animals (1). Another feature of goats by which they differ from other ruminants, is their substantial selectivity towards feeds, so they often choose plants of poorer quality regarding nutrient standards and prefer them at the expense of those of higher nutritive value. Also behavioural problems associated with the domination and subordination of goats may lead to insufficient uptake of feedstuffs by some animals, particularly in herds fed intensively with rich feeds. The authors' studies showed that the factor related to non-typical feeding was excluded. It was confirmed that the digestibility of nutrients by goats is higher compared to other ruminants.

The above mentioned concerns mainly the crude fibre which induces more efficient release of mineral components from the plant structures. While preparing the formulation of the mineral-herbal mixture we focused on the following: beneficial effect of herbs on digestive processes, protective covering of the alimentary tract, stimulation of appetite, relieving stress caused by environmental factors, stimulation of the immune system. The data from the professional literature concerning immune processes in animals indicate that zinc and magnesium have a significant effect on both cellular immunity and humoral immunity (4).

In addition to other effects, these bio-elements participate in the activation of important immunity factors of serum, e.g., properdin, complement, and acidic and alkaline phosphatase. They also affect indirectly the metabolic processes resulting in the synthesis of vitamins, particularly vitamins A, D3 and those from group B that are essential for the immunological processes.

Moreover, the elements contribute to the biosynthesis of nucleic acids and proteins by means of DNA and RNA polymerase.

However, on the other hand, herbal ingredients can inhibit the processes of element absorption as compounds of quite varied structures contained in the herbs can decrease availability of elements, e.g. by formation of chelate compounds among others with copper (7). This may result in variations of behaviour of some micro-elements, although the mineral-herbal mixture examined exerted a positive influence on the mineral metabolism of the kids, especially with regard to Mg, Cu and Fe. And these were the very elements the serum levels of which were investigated in the soil-plant-animal system and were regarded as insufficient for the respective animals.

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THE IMPACT OF THE MINERAL NUTRITION OF COWS ON THE LEVEL OF MINERAL ELEMENTS IN THE HAIR OF THEIR HIDES

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ABSTRACT

The objective of the present study was to evaluate the influence of two mineral preparations on the level of selected elements Ca, P, Mg, Na, K, Fe, Cu, Zn in the hide hair of cows kept on farm A in the south-eastern part of Poland. The animals under study were divided into three treatment groups, twenty cows in each. Group C received no mineral additives, Group D1 was fed rations supplemented with ammonium phosphate — POLIPASZ A (produced by a Chemical plant in Police), and Group D2 received in their rations the mineral-herbal mixture BOVIFOSFOMAG (formulated by the authors). The experiment showed that addition of minerals to cow rations resulted in significantly higher levels of Ca, Mg, Cu and Zn in the hide hair of cows that received BOVIFOSFOMAG supplement and of phosphorus in the group supplemented with POLIPASZ A.

Key words: cows; hair; mineral additives; mineral nutrition

INTRODUCTION

The imbalance of mineral nutrition in ruminants is associated most frequently with bio-geochemical conditions, i.e. transfer of mineral elements from the soil through fodder crops and feedstuffs into animal bodies. Additional problems arise due to individual metabolic-productive responses to deficiencies and disproportions in animals related to the efficiency level of homeostatic mechanisms. The present study investigated the mineral balance in cows kept in a mineral-deficient region and supplemented with mineral mixtures according to formulas developed by the authors considering the bio-geochemical conditions in the area examined.

MATERIALS AND METHODS

The experiment was conducted for two years on a farm "A" situated in the south-eastern part of Poland. The detailed examination included sixty cows of BW breed, four to eight years old, with a mean milk yield of 2550 kilograms, of identical breeding and physiological parameters. The cows were maintained in a conventional alcove-pasture system, fed feedstuffs produced on the farm in compliance with *Farm Animal Feeding Standards* (6) and had *ad libitum* access to drinking water. The animals were allocated to three groups, twenty animals in each. Control group C received a non-supplemented diet, group D1's diet was supplemented with an ammonium phosphate preparation named POLIPASZ A at a dose of 250 grams daily.

The preparation consisted of N — min. 140 $g.kg^{-1}$, P —min. 230 $g.kg^{-1}$, P_2O_5 — min. 540 $g.kg^{-1}$, F — max. 2 $g.kg^{-1}$, Pb — max. 10 $mg.kg^{-1}$ d.m., As — max. 5 $mg.kg^{-1}$ d.m., Cd — max. 3 $mg.kg^{-1}$ d.m., Hg — max. 50 $\mu g.kg^{-1}$ d.m.

The experimental group, D2, received a mineral-herbal mixture named BOVIFOSFOMAG. Its daily dose was 150 grams. The mixture consisted of Ca — 150 $g.kg^{-1}$, P — 80 $g.kg^{-1}$, Mg — 70 $g.kg^{-1}$, Na — 60 $g.kg^{-1}$, Cu — 4 $g.kg^{-1}$, Mn — 15 $mg.kg^{-1}$, I — 8 $mg.kg^{-1}$, Fe — 110 $mg.kg^{-1}$, Zn — 120 $mg.kg^{-1}$, Co — 5 $mg.kg^{-1}$, Se — 4 $mg.kg^{-1}$; vitamin A — 20 000 $IU.kg^{-1}$, vitamin E — 22.00 $mg.kg^{-1}$, vitamin B_1 — 2.00 $mg.kg^{-1}$, vitamin B_2 — 2.00 $mg.kg^{-1}$, vitamin B_6 — 2.40 $mg.kg^{-1}$; nicotinic acid — 15.00 $mg.kg^{-1}$, pantothenic acid — 6.00 $mg.kg^{-1}$, folic acid — 0.40 $mg.kg^{-1}$, Biotin — 0.15 mg, choline — 4.00 $mg.kg^{-1}$.

In addition to this, the following herbs were added partly as a source of vitamins, technologically as carriers and also as substances increasing the palatability of the mixture: flax seed, nettle, chamomile, horsetail, melissa, oak bark and willow bark.

The soil was sampled twice from the pasture and arable land at the height of the vegetation period.

Representative feed samples were collected regularly as the components were introduced into diets. The levels of Ca, Mg, Na, Fe, Cu, and Zn were determined in the soil and feedstuffs.

Hair samples were collected twice from all cows, i.e. one to two weeks before calving and after the first month of lactation and the content of Ca, Mg, Na, Fe, Cu and Zn was determined. The material was sampled according to the recommendations of Brochart (2). The level of elements in the soil, feed and hair was determined by atomic absorption spectrophotometry using a flame spectrophotometer ASA-Unicam 939, and the phosphorus level was determined according to the Fiske-Subbarow method (7).

The results obtained were analysed statistically by ANOVA. The concentrations of elements at every sampling were characterized by an arithmetic mean ($\bar{X}$) and standard deviation (SD). A detailed comparison of the means was made with Tukey's test ($P < 0.05$).

RESULTS

Generally, the reaction of soils was acidic (pH = 6.2). They can be described as poor in P (2.6 ± 0.5 g.kg^{-1} d.m.), Mg (2.0 ± 0.3 g.kg^{-1} d.m.), and Cu (5.4 ± 0.4 mg.kg^{-1} d.m.) and high in available Ca (14.3 ± 2.2 g.kg^{-1} d.m.). The other elements were present in average concentrations (5). The feed samples showed high levels of available Ca (3.8—14.8 g.kg^{-1} d.m.) and deficit of P (0.4—4.6 g.kg^{-1} d.m.) and Mg (0.7—2.1 g.kg^{-1} d.m.).The quantity of Na (1.4—7.1 mg.kg^{-1} d.m.) and K (15.0—41.0 mg.kg^{-1} d.m.) in the feedstuffs may be regarded as optimal. The Cu and Zn levels were low (3) and reached 1.4—7.1 mg. kg^{-1} and 16.0—41.0 mg.kg^{-1}, respectively. The levels of iron, manganese and cobalt were low or reached values considered normal (8). The levels of elements determined in the cow hair are summarised in Table 1.

DISCUSSION

A deficiency of mineral elements in plants is bound to occur only if the soils are especially poor in them (3). In general, soils in Poland are poor in Cu. High Ca levels in the soil decrease concentrations of magnesium, particularly in silage. The synergistic effects of calcium and Cu, Mn and Zn, in plants hinders an explicit interpretation of the results (1, 4). As the content of minerals in hair is considered a helpful index of the mineral supply to an animal body, our investigations focused on the evaluation of the content of minerals in hair. The data presented in Table 1 indicate that P, Mg, Cu and Zn concentration in the group C remained at levels below those regarded as physiological (1).

The opinions on the usability of mineral element determination vary to a great extent. A generally accepted view is that the information about the levels of

Table 1. Mean levels of investigated elements in cow hair [mg.kg^{-1} d.m. (n = 20)]

Element	Sampling	C		D_1		D_2	
		$\bar{X}$ ± SD	P	$\bar{X}$ ± SD	P	$\bar{X}$ ± SD	P
Ca	I	1752 ± 85	A b	1695 ± 45.9	A b	1878 ± 53.1	B b
	II	1665 ± 38.9	A a	1638 ± 61.1	A a	1827 ± 61.5	B a
P	I	173.4 ± 11.15	A a	207.0 ± 13.77	C a	189.4 ± 6.04	B a
	II	166.3 ± 4.57	A a	202.5 ± 5.18	C a	187.4 ± 4.03	B a
Mg	I	365.8 ± 24.74	B b	348.9 ± 6.33	A b	384.9 ± 11.63	C b
	II	327.4 ± 4.23	A a	331.2 ± 8.55	A a	370.7 ± 15.97	B a
Na	I	349.1 ± 14.00	B b	322.6 ± 8.41	A a	367.6 ± 8.91	C a
	II	331.6 ± 11.28	A a	331.7 ± 10.35	A b	357.9 ± 18.41	B a
K	I	1812 ± 120.6	AB b	1748 ± 108.6	A b	1864 ± 80.6	B b
	II	1676 ± 61.7	A a	1646 ± 69.5	A a	1759 ± 80.7	B a
Fe	I	186.8 ± 8.67	A b	187.0 ± 8.34	A b	189.4 ± 6.83	A b
	II	173.3 ± 6.58	A a	169.8 ± 3.41	A a	176.2 ± 9.83	A a
Cu	I	5.78 ± 0.505	A b	5.71 ± 0.412	A b	6.91 ± 0.581	B a
	II	4.92 ± 0.369	A a	5.18 ± 0.393	A a	6.94 ± 0.740	B a
Zn	I	93.9 ± 5.32	B b	89.6 ± 2.78	A a	104.9 ± 3.48	C a
	II	77.8 ± 6.16	A a	86.7 ± 5.05	B a	105.1 ± 2.91	C a

C — control group; D1, D2 — treatment groups

The significance of differences between groups is denoted by upper case letters and that between the samplings with lower case letters; means differ significantly ($P < 0.05$) if not marked with the same letter

mineral elements in hair should supplement that obtained by the examination of blood and milk. This assumption is confirmed by being aware that the accumulation of elements in particular organs and tissues is not uniform, e.g. highest concentrations of zinc are recorded in the eye choroid, bone marrow, teeth, pancreas, gonads and hair (1, 8).

The Ca levels determined in our study were high and exceeded 1500 mg.kg^{-1} d.m. The levels determined in the animals from group D2 were significantly higher than those in groups C and D1. The differences in Ca levels between groups C and D1 determined at two samplings were insignificant. Concentration of P in groups C and D2 was significantly lower compared with D1 throughout the experiment. It indicated that the deposition of minerals in hair was long-lasting and even quite a long interval between the samplings has not resulted in any significant changes in phosphorus concentration in hair of the cows from group D2. It also confirmed the opinion about considerable deficit of P in cows from the examined region (8).

A considerable increase in phosphorus concentration in hair of cows from group D1 must have resulted from a large supply of the element by the diet. The dietary phosphate. Mg level in group D2 was reported to be significantly higher at first and second sampling compared to group D1.

A decreased magnesium concentration in hair in relation to the optimum value, 750 mg.kg^{-1} d.m. (1), implies a deficiency in this macro-element. The supplementation of group D2 resulted in an increased level of this element compared to the other groups, although the increase was insignificant. The same applies to sodium. Its level in the hair of animals from group D2 was significantly higher than that in animals from groups C and D1 in two samplings. The results of the first sampling showed that potassium levels differed significantly between groups D1 and D2. Those in group D2 were significantly higher. The changes should be evaluated considering the interactions between Na and K.

The Fe content in all three groups at first sampling did not differ significantly, yet it was significantly higher than that determined after the second sampling. Variations of Fe concentration in cow hair, i.e. comparison of results in cows in the dry period and during lactation, could be a result of mechanisms that determine the serum level of this element. The Cu content in groups D1 and C did not differ significantly in the two samplings. However, the level of this element was significantly higher in group D2 throughout the experimental period in comparison with its content in groups D1 and C.

The Zn concentration in cows from group D2 was significantly higher in the successive samplings compared to the level of this element in the other groups. Investigation of the levels of Cu and Zn revealed that increasing the supply of these elements causes their faster deposition in hair.

In conclusion, the mineral nutrition of cows favourably affected the serum level of the respective elements.

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THE INDUCTION OF MICRONUCLEI INTO BOVINE LYMPHOCYTE CULTURES EXPOSED TO CYCLOHEXANONE

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ABSTRACT

The aim of this study was to determine the frequency of micronuclei (MNi) in bovine peripheral lymphocytes after exposure to cycklohexanone *in vitro*. The cytokinesis block micronucleus assay (CBMN) for estimating genotoxic activity was used. The results obtained indicated that cyclohexanone induced micronuclei very weakly in bovine peripheral lymphocytes (without S9). A significant elevation of MNi was observed in the concentration of cyclohexanone (0.1 mmol.l^{-1}). The influence of metabolic activation on the genotoxic activity of cyclohexanone was investigated, too. When lymphocyte cultures were treated with cyclohexanone together with a liver membrane fraction (S9) from Aroclor 1254-induced rat liver, the number of micronuclei in binucleated cells increased statistically significantly at concentrations of 5 and 0.5 mmol.l^{-1}, respectively.

Key words: bovine peripheral lymphocytes; cyclohexanone; micronucleus; S9

INTRODUCTION

Cyclohexanone can be released into the environment through the air and waste water emissions involved with its industrial production. If released into the atmosphere, cyclohexanone will degrade relatively rapidly reacting to sunlight-produced hydroxyl radicals. If released into water, cyclohexanone may degrade significantly through biodegradation and photolysis. Humans will be primarily exposed to cyclohexanone by inhalation or dermal contact in occupational settings. The general population may be exposed through consumption of contaminated drinking water or inhalation of contaminated air.

Cyclohexanone is an important industrial compound, which is also useful as a raw material for organic syntheses and is an excellent solvent for most fats, oils, crude rubber, some biomedical polymers and natural and synthetic resins and gums. It is a cyclic 6-carbon ketone, whose formula is $CO(CH_2)_4CH_2$. Some reports suggest cyclohexanone may pose some degree of hazard to exposed individuals, from industrial sources, medical devices or other sources (3). In these cases it has behaved as a primary irritant intra-dermally, dermally and ophthalmically. In general, regardless of the route or species employed, cyclohexanone produces a syndrome of narcosis, tremors, ataxia, hindleg paralysis, hypothermia, respiratory depression and death. Weller and Griggs (10) have demonstrated that exposure of fertilized chicken eggs to vapors of cyclohexanone results in functional abnormalities in the developing chick. Cyclohexanone can alter,too, the cerebrovascular blood flow, which may account, in part, for one of the health symptoms, headaches (7).

However, no work has been reported on the direct effect of cyclohexanone on chromosomes. Because of the widespread use of cyclohexanone and since any attention has been paid to the genotoxicity of the compound, we have evaluated the genotoxicity of this agent in bovine peripheral lymphocytes *in vitro*.

MATERIAL AND METHODS

Cyclohexanone (99.9 %, Ites, The Slovak Republic) was dissolved in re-distilled water and added to the cultures at concentrations of 10; 5; 1; 0.5; 0.1 mmol.l^{-1}.

Lymphocytes cultures were prepared by adding 0.5 millilitres of heparinized whole blood from two healthy donors (Black spotted cattle, six months old) to five millilitres of chromosome medium RPMI 1640 supplemented with L-glutamine, 15

Table 1. The frequency of MNi and cytotoxicity index (% of binucleated cells) in cultured bovine lymphocytes exposed to cyclohexanone: Donor A

Treatment	Concentration mmol.l^{-1}	% BN	Number of MNCB
48 h (-S9)	Control	23.7	21
	10	18.7	28
	5	14.3	31
	1	22.1	31
	0.5	20.2	32
	0.1	18.7	26
MMC		23.9	49***
2 h (+S9)	Control	19.6	23
	10	14.5	24
	5	17.0	42*
	1	16.5	36
	0.5	19.5	39
	0.1	21.1	30
CP		24.9	51***

* — Statistical significance at $P<0.05$
*** — Statistical significance at $P<0.001$

Table 2. The frequency of MNi and cytotoxicity index (% of binucleated cells) in cultured bovine lymphocytes exposed to cyclohexanone: Donor B

Treatment	Concentration mmol.l^{-1}	% BN	Number of MNCB
48 h (-S9)	Control	29.0	19
	10	18.3	24
	5	19.1	23
	1	20.0	27
	0.5	16.5	26
	0.1	19.6	35*
MMC		27.5	48***
2 h (+S9)	Control	22.8	20
	10	21,6	17
	5	17.4	22
	1	14.0	24
	0.5	15.2	35*
	0.1	18.4	24
CP		20.2	48***

* — Statistical significance at $P<0.05$
*** — Statistical significance at $P<0.001$

mmol.l^{-1} HEPES (Sigma), 15 % foetal calf serum, antibiotics (penicillin 250 U.ml^{-1}, streptomycin 250 mg.ml^{-1}), and phytohaemagglutinin (PHA, 180 mg.ml^{-1}, Welcome, Dartford, UK).

Mitomycin C (MMC, Sigma, St. Louis, MO, USA, 0.4 µM), and cyclophosphamide (CP, Jenapharm, Ankerwerk, Rudolstadt, Germany, 100 µM) were used as positive control agents in the assays in the absence and presence of the metabolic activation (S9 mix).

For the MN assay the bovine cultures were incubated at 37 °C for 72 h and, forty-four hours from the initiation, cytochalasin B (Cyt. B, Sigma, St. Louis, MO, USA)) at a final concentration of 6 µg.ml^{-1} was added to arrest cytokinesis.

Some genotoxic chemicals can produce their effects directly, whereas others require metabolism to mutagenically active intermediates (7). Aroclor 1254-induced rat liver homogenate supernatant (S9) is routinely used as an exogenous metabolic activation system for the evaluation of mutagenicity of xenobiotics (1).

The cultures treated for two hours with S9 mix (four millilitres of chromosome medium with 0.5 millilitres of S9) and those without S9 mix were set up without heat inactivated foetal calf serum. After treatment, the cultures were washed twice with PBS and reconstituted in the same way as those cultures treated for forty-eight hours. Slides were obtained by the standard cytogenetic method.

Classifying 500 cells according to the number of nuclei was evaluated in terms of toxicity. The well-known cytotoxicity index was used: percentage of binucleated cells (% BN).

The induction of MN was evaluated by scoring a total of 1,000 binucleated cells with well-preserved cytoplasm for each animal and each concentration.

The statistical analysis of results was performed using a χ^2-test to estimate the induction of MNi.

RESULTS

Tables 1 and 2 show frequencies of micronuclei (MNi) and the cytotoxicity index (% of binucleated cells) obtained after treatment with cyclohexanone. In each table, the data obtained from the different experimental conditions are shown: treatments lasting forty-eight hours without microsomal fraction and treatment for two hours with S9 microsomal fraction, respectively. The positive controls used were MMC (0.4 µM) in the experiments without microsomal activation and CP (100 µM) in the experiments with S9. Cyclohexanone treatments lasting for forty-eight hours appear to induce a very slight but statistically significant increase in MNi frequency in cultures at the lowest concentration tested (0.1 mmol.l^{-1}).

The results from the experiments for two hours in the presence of S9 fraction showed a slight but significant increase in the MN level at the concentrations tested (5 and 0.5 mmol.l^{-1}, respectively).

We used one of the known cytotoxicity indexes: the percentage of binucleated cells (2). From our cytotoxicity data is obvious that the percentage of BN cells did not reflect the reduction of cell proliferation.

In conclusion, our results indicate that cyclohexanone is able to exert a weak effect on frequency of micronuclei in bovine peripheral lymphocytes.

DISCUSSION

To our knowledge, there are no reports available describing the cytotoxicity or genotoxicity effects of

cyclohexanone on domestic animal cells. In the present study, the ability of cyclohexanone to induce genetic damage was evaluated in the cytokinesis block micronucleus (CBMN) assay. This technique is considered to be a reliable method for the detection of micronuclei, mainly after improvement according to Fenech and Morley (2), using cytochalasin B to arrest cytokinesis and discriminate between undivided cells and those that undergo one or more divisions. The CBMN assay appears to be sensitive enough to detect both clastogenicity and aneuploidy.

One important application of cyclohexanone is as a solvent sealer for polyvinyl chloride (PVC), which is used in a number of medical devices (5). In this use, it is possible for residual cyclohexanone to remain in the device and to be leached from it by the container solution.

Ulsaker and Korsnes (9) found cyclohexanone concentrations ranging from 2.9 to 15.9 $\mu g.ml^{-1}$ in intravenous solutions stored in PVC bags. Thus, the potential exists for inadvertent clinical administration of small amounts of cyclohexanone that have leached into parenteral solutions.

From the results of the study by Gupta *et al.* (3) it seems evident that repeated doses of cyclohexanone produced a significant cumulative toxic effect. Thus the possibility of cyclohexanone being administered to patients, and the paucity of existing information, provided the impetus for additional studies also on the genotoxicity of the compound.

According to data by Lijinsky and Kovatch (6) by oral administration of cyclohexanone in the drinking water of one strain of mice and one strain of rats there is convincing evidence of carcinogenicity. In mice B6C3F1 there was a slight increase of tumours that occur commonly in this strain only at low dose. In rats F344 there was a slight increase of adrenal cortical tumours only in males treated with the low dose. The authors suggested that the carcinogenic activity of cyclohexanone is marginal and the effect, if any, is weak.

In another study it was found by Samini *et al.* (1985) that inhalation exposure to cyclohexanone vapour in pregnant rats was unlikely to be developmentally toxic.

In 1999 cyclohexanone was registered by IARC (4) like that: *"Not classifiable as a human carcinogen (Group 3)"*.

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A CASE REPORT OF *Encephalitozoon cuniculi* IN THE NEDERLAND DWARF BREED OF *Oryctolagus cuniculus* IN SLOVAKIA

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SUMMARY

Encephalitozoonosis is characterised as an infection in rabbits with a prevailingly chronic asymptomatic course of a disease. There are a growing number of cases with the occurrence of clinical neurological signs manifesting themselves as *thorticollis*, *opisthotonus*, circumvolute movements, loss of weight and asthenia of the hind-limbs, all of them pointing to an encephalitozoonosis. Recently there has been increasing interest in domestic rabbit breeding, especially in the breed Nederland Dwarf of *Oryctolagus cuniculus*. Our case report is the first communication about the spontaneous encephalitozoonosis of this breed of rabbits in Slovakia. In order to diagnose the pathogenic agent of the disease, both methods – the indirect immunofluorescence test (IFAT) based on the detection of specific antibodies to *Encephalitozoon cuniculi* in the serum of the rabbit and the direct visualisation of spores in the faeces samples of the infected rabbit by means of an optical brightener Calcofluor White M2R – were used. In Slovakia, the spores of *E. cuniculi* were first isolated from the kidneys and brain of an animal and subsequently cultivated in the cell line RK 13.

Key words: Calcofluor White M2R; cell line RK 13; *Encephalitozoon cuniculi;* IFAT; Nederland Dwarf breed; rabbit spontaneous encephalitozoonosis

INTRODUCTION

Encephalitozoon cuniculi is an obligate intracellular protozoan parasite belonging to the phylum *MICROSPORA*. It is pathogenic for both free-living and farm rabbits and other mammals in which it can bring on a chronic, usually latent disease (9). At the present time, there is an increase in interest in keeping the Nederland Dwarf breed of *Oryctolagus cuniculus* in households with small children. Furthermore, the importance of this disease was emphasized after the recognition of the zoonotic character of microsporidiosis (2) and after a number of cases of organ and systemic opportune infections were observed in immuno incompetent people.

Lately, there are a growing number of cases of rabbits (breed Nederland Dwarf of *Oryctolagus cuniculus*) with the occurrence of clinical neurological signs manifesting themselves as *thorticollis*, *opisthotonus*, circumvolute movement, loss of weight, and asthenia of hind-limbs, all of them pointing to an encephalitozoonosis.

It is well known that *E. cuniculi* is an opportune pathogen; therefore, the diagnosis of clinically manifested disease is basically an indicator of inborn or acquired immune deficiency of an afflicted individual. In 1998 the clinical form of the encephalitozoonosis, which developed five weeks after the application of immunosuppressive substance – cyklofosfamid was described by Horváth *et al.* (4). What causes the clinical signs in these rabbits remains debatable as they are bred on farms in good hygienic conditions. Contacts with other animals are restricted and preventive inoculation against all prescribed infections is fully provided for. Among the possibilities of immune system deficiency of an organism may be such impacts as crossbreeding, stress of an individual resulting from frequent manipulation of the owner with the animal or possibly a hidden infection.

Our goal was to isolate and identify the pathogenic agent of encephalitozoonosis.

Fig. 1. Clinical signs in a rabbit: *thorticollis* and asthenia of hind-limbs

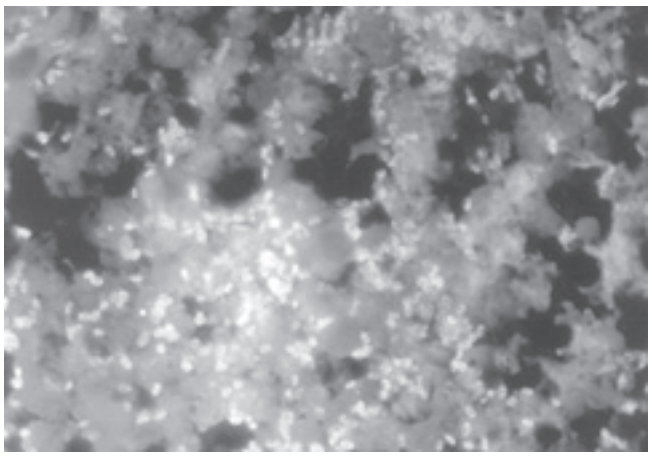

Fig. 2. The spores *E. cuniculi* in the faeces of the examined rabbit. The spores were visualised with the optical brightener Calcofluor White M2R. Green fluorescence (× 1000)

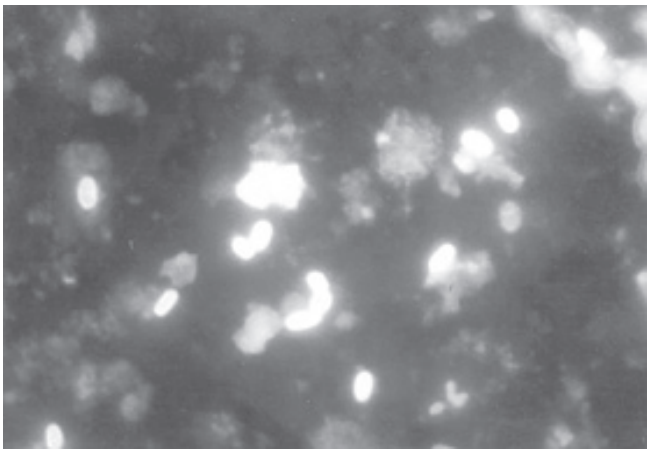

Fig. 3. A positive IFAT examination of the parasite spores after the binding of specific antibodies present in the sera of an examined animal (× 1200). Green fluorescence

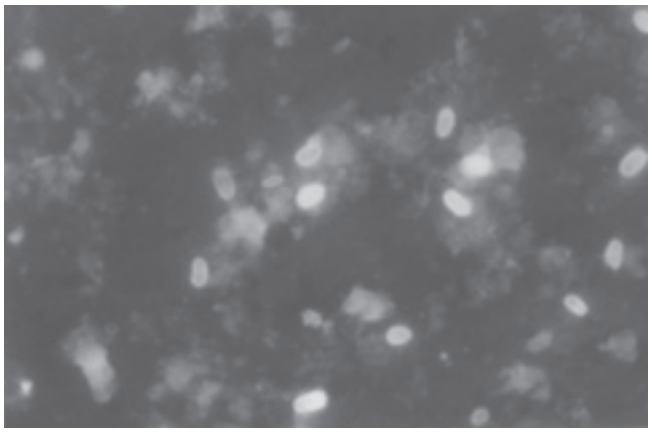

Fig. 4. The spores of *E. cuniculi* were obtained after five weeks of cultivation on a cell line RK 13. The spores were visualised with the optical brightener Calcofluor White M2R. Green fluorescence (× 1200)

MATERIAL AND METHODS

Animal. A domestic rabbit, the breed Nederland Dwarf of *Oryctolagus cuniculus,* with a body weight of 1.25 kilograms was used in the experiment.

Spores. The spores of *E. cuniculi* obtained from the permanent infected cell line RK 13 (rabbit kidney cells) were used as an antigen in serological tests (15).

Antibody. Swine anti-rabbit antibodies conjugated with FITC (fluorescein isothiocyanate) at the titrate 1 : 64 (Sigma, Germany) were used as a specific antibody conjugate.

Direct detection of spores in the faeces sample. The detection of *E. cuniculi* spores with optical brighteners in faeces according to Vávra *et al.* (17) was used. Then they were examined by fluorescent microscope ZEISS JENALUMAR with 405–490 nm excitation filters, 510 nm colour rays and 550 nm barrier filter (green fluorescence) and with 390—420 nm excitation filters, 450 nm colour rays and 470 nm barrier filter (blue fluorescence).

Serological diagnosis by means of the indirect immunofluorescence antibody test (IFAT). The blood sample of a rabbit for serological examination was taken from the vena *auricularis marginalis.* To detect specific antibodies to the antigen *E. cuniculi* an indirect immunofluorescence test (IFAT) according to Chalupsky *et al.* (5) was used.

Isolation of spores of E. cuniculi. The samples of the parts of kidney and brain were taken from a rabbit *post mortem* in sterile conditions and were cut up and crushed in a mortar. Isolation of spores of *E. cuniculi* was used according to Shadduck *et al.* (12).

Cultivation of E. cuniculi. 0.5 ml of prepared isolate was laid on the cell line of RK 13. The cultivation itself was performed in short term culture bottles (25 cm^2) stored in a thermostat with the temperature of 37 °C.

RESULTS

Suspect encephalitozoonosis was diagnosed in one rabbit of the breed, Nederland Dwarf of *Oryctolagus cuniculus* on the basis of clinical signs manifested as *thorticollis, opisthotonus,* circumvolute movements and asthenia of hind-limbs (Fig. 1).

The diagnosis was supported also by the findings of spores in the faeces of the examined rabbit. The spores were visualised with the optical brightener Calcofluor White M2R and identified as bright fluorescent figures 1.5 × 3 mm in size (Fig. 2).

The presence of specific antibodies to *E. cuniculi* was confirmed by the indirect immunofluorescence test. The positive serum served as a guarantee of positive results. The serum of the rabbit reacted in a titrate 1 : 512 with a bright peripheral fluorescence of spores (Fig. 3).

Additionally, after the manifestation of clinical signs, detection of spores in the faeces and the serological examination, the spores of *E. cuniculi* were isolated *post mortem* from the brain and kidneys of the rabbit. The first spores of *E. cuniculi* were obtained after five weeks of cultivation in a cell line RK 13. They were stained with an optical brightener Calcofluor White M2R and identified as light blue oval figures 1.5 × 3 mm in size with a fluorescent microscope using green fluorescence technique (Fig. 4).

DISCUSSION

Encephalitozoonosis was first found in rabbits in USA by Wright and Craighead in 1922 (20) as infectious encephalomyelitis causing motor paralysis in young rabbits. Although the pathogenic agent of the disease — *Encephalitozoon cuniculi* — can be detected in ontogenetically different parenchyma, in fact, its life cycle occurs only in particular types of cells (epithelial, endothelial, and monocytes). *E. cuniculi* has been successfully isolated several times in single mammalian species. Authors have gradually reported the news of its isolation in rabbits (10), mice (16), dogs (11), monkeys (21), humans (18) and young foxes (7). The pathogenic agent of encephalitozoonosis has not yet been isolated in Slovakia however its prevalence has been known since 1995 on the basis of serological, pathomorphological and immunohistochemical diagnostic methods (3, 6, 13).

Stages of the life cycle of microsporidia in rabbits can be identified in various tissues of the majority of organs with a predilection in the brain and kidneys (20). Therefore, these two organs were selected for the isolation of spores.

A variety of direct and indirect diagnostic methods are used in the diagnosis of microsporidian infections in animals. Direct diagnostic methods are facilitated by the fact that spores are Gram-positive and can be easily identified by fluorescent optical brighteners binding their chitinous layer of endospore. The method of direct identification of spores in the stool samples of animals by means of optical brighteners is the simplest and with the acquirement of particular skills, also a precise examination. The detection of microsporidian spores with optical brighteners is easy, quick and reliable and it presents the first step in the diagnosis of microsporidia in humans and animals (17).

The serological method IFAT applied in our experiment belongs to the methods known for both their advantages and disadvantages. The advantage of the majority of serological methods is that, serologically, it is possible to detect specific serum antibodies at least two weeks before the histological finding of the parasite in pathological lesions and at least four weeks before the possible detection of its spores excreted in the urine of infected rabbits (1). Their disadvantage is, chiefly, the fact that an early humoral immune response to *E. cuniculi,* particularly in rabbits, is given mainly by the antibodies of the IgM type; therefore, it is quite likely that positive individuals in early stages of infection will not be detected (19).

The cultivation of *E. cuniculi* is another method that was used in our experiment. The first attempts of the *in vitro* cultivation of microsporidian species appeared in 1934 when Trager (14) tried for the first time to cultivate microsporidia in the insect *Nosema bombycis*. It was almost twenty-two years until cultures of microsporidia were cultivated in mammals. In 1956 Morris, McCrown and Blount (8) cultivated *E. cuniculi* in the murine cell line of lymphosarcoma MB III, however, only for a very short time period. The interest in the cultivation of these parasites was stimulated by Shad-duck in 1969 (10) that successfully produced a long-term cultivation of *E. cuniculi* in the rabbit cell line RK 13. Until 1990 *E. cuniculi* was the only mammalian microsporidium cultivated *in vitro* by a number of researchers either on a long- or short-term basis in a variety of cell cultures. Among the most frequently used cell cultures are such as primary rabbit and monkey kidney cells (RK 13 and VERO E6) and several other cell lines (15, 16). MEM or RPMI supplemented with 5 to 10 % BOFES, antibiotics, and antimycotics (STM, PNC, AFTC) are the most frequently used basic media.

As the importance of this disease was emphasized after the recognition of the zoonotic character of microsporidiosis it is important to isolate, to identify and to know the causal organism of this disease.

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RECIPROCAL TRANSLOCATION IN A CASE OF THE BASAL CELL TUMOUR OF A CAT
(A Short Communication)

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ABSTRACT

A reciprocal translocation (tB1p/C1q) was observed in a cytogenetic investigation of a basal cell tumour in the skin of a female cat. The chromosome aberration was present in a minor fraction (8 %) of the analysed cells.

Key words: basal cell; chromosome; feline; translocation; tumour

Basal cell tumours are common in dogs and cats, but rare in other species. They show a great variety of histological patterns. Solid, garland- or ribbon-like, medusoid, adenoid, cystic and basosquamous varieties, sometimes with foci of keratinization, occur singly or in combination. Mitotic figures and melanin pigmentation are frequently encountered in basal cell tumours (12, 2).

Data characterising feline tumours by cytogenetic banding techniques are rare (4, 5, 6, 7, 8). Our present case reports on a cytogenetic investigation of a basal cell tumour of a cystic pattern in a cat (Fig. 1).

The patient, a 10-year-old neutered female cat, developed a tumour (diameter, 4 cm) in her skin on the left metatarsus. Tumour tissue from the cat was minced into small fragments ($< 1\ mm^3$), which were set up as explant cell cultures (i.e., they were not subjected to an enzymatic suspension step). After thirteen days, the cells were harvested; chromosome staining was done by Giemsa-banding (G-banding) after Wang and Fedoroff (11) and chromosome nomenclature was performed according to Ford *et al.* (1).

Fifty cells were karyotyped. Four cells (8 %) possessed an reciprocal translocation between the short arm of chromosome B1 and the long arm of chromosome C1 (tB1p/C1q); (Fig. 2). The other forty-six cells possessed the normal karyotype and the normal chromosome number (2n = 38) was present in all cells.

To date, only one case of chromosomal aberration of a feline basal cell tumour has been reported (5). This case concerned a monosomy of chromosome E3. However, this tumour was a basal cell carcinoma showing a solid pattern.

Clearly, it would be too early to allow a preferential assignment of certain chromosome aberrations to distinct types of basal cell tumours. Currently there are only very few loci oncogenes and tumour suppressor loci known to be localized on chromosome B1 and C1, e.g. kit, jun and N-ras in the feline genetic map (3, 9, 10). Considering the limited cytogenetic and molecular genetic data, no firm conclusions can be drawn about the impact of the observed translocation in the genesis and progression of feline basal cell tumours until now. However, such novel results may become important for future veterinary and comparative tumour medicine.

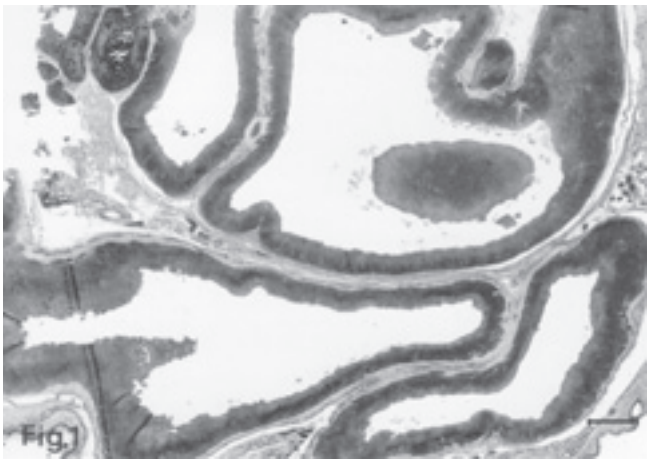

Fig. 1. Basal cell tumour µm of a cat, cytic pattern. Bar represents 600 µm

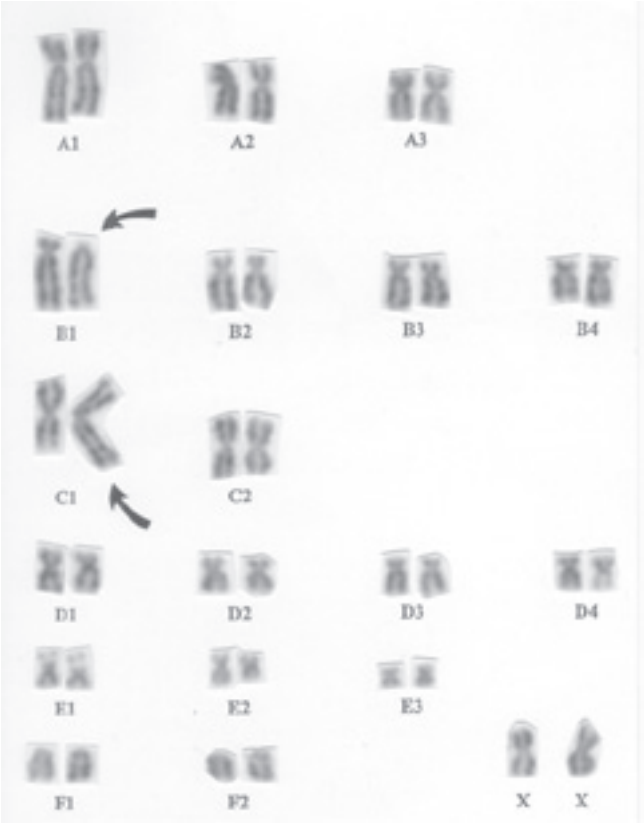

Fig. 2. Trypsin G-banded karyotype (2n = 38). The arrows indicate the two involved chromosomes of the reciprocal translocation (tB1p/C1q)

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CHRONICLE

MVDr. Miloš Halaša, CSc. (1923—2002)

MVDr. Miloš Halaša, a long-time director of the Central State Veterinary Institute, organizer and builder of laboratory and clinical diagnostics in Slovakia.

He was born on 11th July, 1923, in the village Záskalie, today part of Dolný Kubín. He was born into a large family of smallholding peasants in Orava. Already in his early years he radiated kindness and sensitivity, the gifts given by Orava's harsh nature to its children at their birth. Because of this it has been said that people from Orava are faithful friends.

He obtained his primary and secondary education at the State secondary school in Dolný Kubín where he passed his school-leaving examination in 1943. In the same year he entered military service. Although these were very difficult years he easily found, together with thousands of other Slovaks, his right place in this cataclysm. He took part in the Slovak National Uprising and in 1945, as a member of the 1st Czechoslovak Army Corps, also in the combat for freedom in the Liptov region.

After the war he decided to study veterinary medicine at the College of Veterinary Medicine in Brno. It has been said that if a man from Orava has to leave his native soil the nostalgic feelings for his countryside, a hearty attitude to work and zeal for the best in life are ideals that remain with him forever. In the Moravian metropolis he obtained not only professional education but also experience in life for use in future years. He successfully traversed this important period of his life and on the 15th November, 1949, obtained a veterinary diploma. He defended his dissertation thesis on the theme *"Trichomonas foetus, its Isolation and Cultivation in vitro"* and was graduated as a veterinary doctor on the 21st January, 1950. Already during his studies in Brno he had been employed as a trainee assistant at the Department of Biology and Parasitology. There he acquired an inclination for research activities. His work at this Department affected his further professional direction during veterinary practice.

After completion of his college studies (1945—1950) he became a member of the staff of the Central Office of the Czechoslovak State Farms in Prague and in the period from 1951 to 1952 he was the head of veterinary services at the Regional Centre of State Farms in Bratislava. From 1952 to 1953 he worked for the State Veterinary Administration of the Comission for Agriculture in Bratislava. While working there he was involved in the problems of artificial insemination. Although the field work was interesting and relatively diverse it did not satisfy him fully and because of this he made an effort to apply his professional knowledge and experience gained at the parasitological department of the Veterinary College in establishments of similar orientation and become involved in laboratory and research activities.

His dream became true in 1953 when he was accepted by the State Scientific Veterinary Institute (SSVI) in Bratislava (Regional Institute for Slovakia) as the head of the Department of Parasitology. After the transformation of the Institute to a branch office of SSVI in Prague he was appointed director of this workplace from the 1st March, 1958 (replacing the recalled Dr. Nižnánsky). In 1969, after various organisational changes, he became director of the newly established Central State Veterinary Institute (CSVI) and worked in this capacity up to 1984. This post allowed him to make full use of his

professional knowledge and managing abilities. He succeeded in complying with the request of Dr. Samuel Adamaťˇ, the head of the State Veterinary Service, presented on February 14, 1949, at the national meeting of Slovak veterinarians in Bratislava, to establish diagnostic institutes in all regions of Slovakia.

Dr. Halaša, as a long-time director of SSVI, contributed considerably to the development of laboratory and clinical diagnostics in Slovakia. It was not easy work as it was constantly necessary to adjust to new conditions and requirements of practice. In addition to the introduction of new scientific knowledge into diagnostics, it was also necessary to change its character as it was inevitable to change the entire veterinary services. This required a great deal of organisation, conceptual and specialised work and the ensuring of the completion of already existing and creation of new workplaces all-over Slovakia; equipping them with laboratory equipment and ensuring the training of specialised personnel.

It is necessary to stress the contribution of Dr. Halaša and the staff of diagnostic institutes to the implementation of plans for the improvement of the health of animal herds and the elimination of dangerous diseases. Extensive preventive examination of animals prevented not only the introduction of serious diseases but also stabilised the infectious situation. The elimination of neither tuberculosis nor brucellosis was possible without laboratory diagnostics. The diagnostic institutes played an important role also in the area of hygiene-health inspection that covered the obtaining, processing and distribution of raw materials and food of animal origin.

In 1972, under the leadership of Dr. Halaša, the CSVI in Bratislava worked out a long-term concept of the development of laboratory and clinical diagnostics in Slovakia based on the specialisation of individual institutes according to branches of science and the animal species. The specialised institutes became consultation centres for resolving veterinary medical problems affecting individual animal species. Dr. Halaša was the head of the Reference Laboratory for Diagnostics of Leptospirosis. He concentrated on these problems from 1954 until 1988 and became a renowned specialist in this area. He collaborated closely with a number of medical workplaces. Part of the knowledge obtained was compiled in his dissertation CSc. thesis on the theme *"Laboratory Diagnostics, Dissemination and Epizootiology of Swine Leptospirosis in Slovakia"*. Additional results were published in specialised papers and books.

In addition to organising and managing activities he paid considerable attention to research work. He focused particularly on parasitic diseases. This is testified to by numerous papers and final reports of partial research tasks and concrete stages and active editorial activities. He published more than one hundred scientific and specialised papers in journals at home and abroad.

He was author or co-author of five books, namely: Halaša, M. *et al.*: *"Leptospiroses of animals and humans"* (Bratislava, 1969), Gdovin, T., Kouba, V. *et al*.: *"Diseases of pigs"* (Prague, 1966), Dražan, J. *et al*.: *"Infectious Diseases of Farm Animals,* Part I: *Bacterial and protozoan diseases"* (Prague, 1967), Havelka, B., Halaša, M., Vasiľ, M.: *"Inflammation of mammary glands of dairy cows"* (Bratislava, 1975), Haladej, Š. *et al.: "25 Years of Socialistic Veterinary Medicine"* (Bratislava, 1976).

He held several important functions. He was the head of the Reference Laboratory for Leptospirosis with nation-wide activities, a long-time member of the Editorial board of journal *Veterinářství* and *Proceedings of SVA SSR*, a member of examining commissions for final state exams at the College of Veterinary Medicine in Košice, of the Scientific Board of the Research Institute of Veterinary Medicine (RIVM) in Brno, Research Institute of Veterinary Medicine in Košice, and Research Institute of Preventive Medicine in Bratislava, the commission of the Governmental Presidium of SSR for Dealing with Diseases Transmissible from Animals to Humans and also a member of the Veterinary Medicine Branch of CzSAZ and chairman of its Commission for Zoonoses.

He was a long-time member of coordination commissions for research tasks within veterinary organisations in the former ČSSR. He participated himself in work on many research projects or their stages concerning leptospirosis infections, invasive diseases and mycoses included in the state, branch, institutional or "Z" research plans.

He took part in the post-graduate education of veterinarians. He frequently read lectures at scientific conferences, symposia and seminars throughout the republic. He participated in the practical utilization of research project outputs as they were worked on and presented by Czechoslovak veterinary research establishments, for example RIVM Brno, RIVM in Košice and Colleges of Veterinary Medicine in Košice and Brno.

Dr. Halaša was one of the personalities who in the second half of the twentieth century contributed considerably to the development of our veterinary services. His long-time work involvement was very intensive and purposeful. Throughout his life he could rejoice over work well executed, but was not lulled into false self-satisfaction and retained his highly demanding approach and precision.

Dr. Halaša was an acknowledged personality involved in the laboratory part of veterinary services that built upon a solid basis capable of fulfilling demanding tasks in both areas, diagnostic and research. He met regularly with the members of staff of diagnostic institutes throughout Slovakia and gave them all possible help and directed their professional growth. He could appreciate scrupulous professional work of his colleagues. He was very disturbed over the abolition of some diagnostic institutes during the last years of his life.

He enjoyed memories of his work in Prague where he met his solicitous wife who followed him through life and produced a serene family environment around him.

He returned frequently to his native Orava where he met with his compatriots and friends. He took delight in the wonders of Orava nature and found there inspiration for his future work.

The results of his life-long work were marked by many awards and medals. He was awarded the following: Medal for Valour (1945), Order of SNU of the IInd degree (1946) Commemorative Medal of SNU (1964). Medal of Partisan Brigade of General Štefánik (1974). He was also awarded two state, three branch, and three trade union distinctions. Additional honours: Gold Medal of Prof. P. Adámi; Honorary Bronze Medal of CzSAZ (1983).

Distinctions awarded abroad: Order of Cuban Revolution (1980), honorary membership of the Parasitological Society of Cuban Republic (1987).

Even after retiring for several years he worked with the staff of the State Veterinary Administration as an expert for keeping and diseases of bees when he was able to use his long-time breeding and professional experience. He participated in the preparation of proposals of veterinary regulations and specialised texts on the theme of bee diseases. In the last years of his life he became interested in the history of veterinary medicine. He was the head of the team of authors who prepared the publication *"60 Years Of Veterinary Laboratory And Clinical Diagnostics In Slovakia"* and participated also in compiling biographies of some important personalities in veterinary medicine.

He died suddenly on the 29th September, 2002. He was buried in Bratislava on the 4th October, 2002, mourned by members of his family, friends, acquaintances and many veterinary circles.

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