FOLIA VETERINARIA

The scientific journal of the
UNIVERSITY OF VETERINARY MEDICINE AND
PHARMACY IN KOŠICE — The Slovak Republic

ISSN 0015-5748

1

LVIII • 2014

EDITORIAL BOARD

FOLIA VETERINARIA is issued by the *University of Veterinary Medicine and Pharmacy in Košice (UVMP);* address: Komenského 73, 041 81 Košice, The Slovak Republic (tel.: +421 915 984 669, fax: +421 55 632 52 93, E-mail: Milada.Vargova@uvlf.sk).

IČO: 397 474

The journal is published quarterly in English (numbers 1—4) and distributed worldwide.

Subscription rate for 1 year is 120 €. Orders are accepted by *The Department of The Scientific Information — The Library of The University of Veterinary Medicine and Pharmacy in Košice* (UVIK), E-mail: Natalia.Palencarova@uvlf.sk; the subscription is accepted by the State treasure.

Bank contact: State treasure, Radlinského 32, Bratislava 15, The Slovak Republic; **account number:** 7000072225/8180.

Issued on March 25, 2014

FOLIA VETERINARIA, vydáva *Univerzita veterinárskeho lekárstva a farmácie v Košiciach (UVLF),* Komenského 73, 041 81 Košice, Slovenská republika (tel.: 0915 984 669, fax: 055/632 52 93, E-mail: Milada.Vargova@uvlf.sk).

IČO: 397 474

Časopis vychádza kvartálne (č. 1–4) a je distribuovaný celosvetove.

Ročné predplatné 120 €. Objednávky prijíma *Ústav vedeckých informácií a knižnice Univerzity veterinárskeho lekárstva a farmácie v Košiciach* (UVIK), E-mail: Natalia.Palencarova@uvlf.sk; predplatné Štátna pokladnica (na nižšie uvedené číslo účtu).

Bankové spojenie: Štátna pokladnica, Radlinského 32, Bratislava 15; **číslo účtu:** 7000072225/8180.

Dátum vydania: 25.3.2014

Tlač: **Univerzita veterinárskeho lekárstva a farmácie** Komenského 73, 041 81 Košice

Sadzba: **Sapfo publishers**, Szakkayho 1, 040 01 Košice

EV 3485/09

For basic information about the journal see Internet home pages: www.uvm.sk; www.uvlf.sk

Indexed and abstracted in AGRIS, CAB, EBSCO

FOLIA VETERINARIA

PUBLISHED BY
THE UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE
THE SLOVAK REPUBLIC

Folia Veterinaria
Vol. 58, 2014

VYDÁVA
UNIVERZITA VETERINÁRSKEHO LEKÁRSTVA A FARMÁCIE V KOŠICIACH
2014

FOLIA VETERINARIA, 58, 1, 2014

CONTENTS

ABEBE, T., ASEFA ASMARE, A., BELAY, M.: SEROPREVALENCE OF BRUCELLOSIS IN MALE CAMELS (*CAMELUS DROMEDARIUS*) FROM BORENA AND BALE AREAS, ETHIOPIA 5

GAJDOŠÍK, M., KOZÁNEK, M., JR., TAKÁČ, P.: MAGGOT DEBRIDEMENT THERAPY FOR SUCCESSFUL HORSE WOUND TREATMENT (A CASE REPORT) 9

HOLOVSKÁ, K., ALMÁŠIOVÁ, V., CIGÁNKOVÁ, V., PETROVOVÁ, E.: STRUCTURAL AND ULTRASTRUCTURAL STUDY OF OVAL CELLS IN RABBIT'S LIVER AFTER BENDIOCARB ADMINISTRATION 15

CAPÍK, I., ORSZÁGHOVÁ, A., KREMEŇ, J.: PLATELET-RICH PLASMA, BIOLAMP AND LASER IN SKIN GRAFT HEALING 21

ALMÁŠIOVÁ, V., HOLOVSKÁ, K., CIGÁNKOVÁ, V., TARABOVÁ, L., BEŇOVÁ K., RAČEKOVÁ, E.: EFFECT OF PULSED ELECTROMAGNETIC RADIATION ON THE RAT TESTIS AND EPIDIDYMIS: HISTOLOGICAL STUDY 25

BAČA, M., MARCINČÁK, S., ČERTÍK, M., ADAMECHOVÁ, Z., NEMCOVÁ, R., GANCARČÍKOVÁ, S., POPELKA, P., MARCINČÁKOVÁ, D.: PROFILE OF FATTY ACIDS AND LIPID OXIDATION IN PORK AFTER FEEDING LINSEED AND VITAMIN E 31

MIHALOVÁ, M., TRBOLOVÁ, A.: IS THERE A RISK OF HEREDITARY EYE DISEASES (HED) IN SLOVAK NATIONAL DOG BREEDS? (A REVIEW) 37

MAŽENSKÝ, D., PETROVOVÁ, E., SUPUKA, P., SUPUKOVA, A.: CORROSION TECHNIQUE IN THE STUDY OF ARTERIAL VARIATIONS AS THE RESULT OF A CROSS OF THE SLOVAK GREY-BLUE REX AND BLUE OF VIENNA RABBITS 43

VÁCZI, P., ČONKOVÁ, E., MÁRTONOVÁ, M., SIHELSKÁ, Z.: EFFICACY OF ANTISEPTICS AGAINST THE YEAST *MALASSEZIA PACHYDERMATIS* 47

GUIDANCE FOR CONTRIBUTORS 53

FOLIA VETERINARIA, 58, 1: 5—8, 2014

SEROPREVALENCE OF BRUCELLOSIS IN MALE CAMELS (*CAMELUS DROMEDARIUS*) FROM BORENA AND BALE AREAS, ETHIOPIA

Abebe, T.[1], Asefa Asmare, A.[2], Belay, M.[1]

[1]Wollo University, P. O. Box 37, Dessie
[2]Wolaita Sodo University, P. O. Box 138, Sodo
Ethiopia

asefa62asefa62@gmail.com

ABSTRACT

A total number of 1500 apparently healthy male camels were sampled during the period from December 2011 to April 2012 to determine the seroprevalence of brucellosis in these male camels. Out of these animals, 1149 were brought from Borena, Ethiopia and 351 camels from Bale, Ethiopia areas. Blood samples were collected from all animals and the sera were separated. All serum samples were initially screened for the presence of *Brucella abortus* antibodies by the Rose Bengal Plate Test (RBPT) and positive reacting sera were confirmed by the Complement Fixation Test (CFT). The overall prevalence of brucellosis in camels in this study was 0.5 %. The seroprevalence of brucellosis in the male camels from the Bale area (0.6 %) was slightly higher than in the male camels from the Borena area (0.5 %). There was no significant difference in the prevalence of brucellosis in male camels brought from Borena (0.5 %; n = 6) and Bale areas (0.6 %; n = 2) as confirmed by the CFT ($X^2 = 0.011$; df = 1; p = 0.915). All RBPT brucellosis seropositive male camels from the Bale area were also found to be seropositive by the CFT, whereas of the 9 (0.8 %) RBPT brucellosis seropositive male camels from the Borena area, 6 (0.5 %) were confirmed to be brucellosis seropositive by the CFT. The presence of *B. abortus* antibodies in all age groups was equal (G1 = 0.5 %; G2 = 0.5 %; G3 = 0.5 %; $X^2 = 0.00$; df = 2; p = 1.00). The result of this study revealed that brucellosis occurs in male camels from Borena and Bale areas. *Brucella* organisms that affect camels are virulent for humans and also have economic importance mainly by affecting the camel export market. Therefore, camel brucellosis needs much more attention.

Key words: *Brucella abortus*; Complement Fixation Test; male camel; Rose Bengal Plate Test; serum

INTRODUCTION

The camel (*Camelus dromedarius*, one-humped camel) is an important livestock species uniquely adapted to arid and semiarid environments. It was probably domesticated in present day Africa [19]. According to Schwartz and Dioli [19], two-thirds of the world and over 80 % of the African camel population inhabit the arid areas of Somalia, Sudan, Ethiopia, Kenya, and Dijibuti. About 11.5 million camels reside in this region. Ethiopia takes the 3rd place in Africa next to Somalia and Sudan in possessing over 1.5 million camels [5]. In Ethiopia, camels are reared in the arid and semiarid areas of the Northeastern, Eastern, and Southeastern parts of the country; mainly in Borena, Somali, Afar, and Bale regions by pastoralists and agro-pastoralists [15].

The unique ability of camels to adapt to harsh environments enables them to survive in extremely arid areas. The most significant aspect of this adaptation is the economic use of water in almost all metabolic aspects. Camels can survive 15 to 20 days without any water [18]. Camels also adapt to fluctuations in forage quality by either increased selectivity for high quality plant material or by more efficient digestion of poor quality materials [9], [17], [18].

Camels are extremely important for the livelihood of the pastoralist communities and their cultural life [5], [13], [20]. They provide milk, meat, wool, hair and hides, serve for riding, as pack animals and as draft animals for agriculture, and for short distance transport [19] in areas where feed and water resources are scarce. Live weights of 650 kg in females and over 800 kg in males are not uncommon [18]. Daily milk yields of camels range between 3 and 6 litres, with total yields between 1500 to 2500 litres produced within a lactation period of 15 to 18 months. Ethiopia produces 174,000 tons of camel milk and 20,000 tons of camel meat per year [19]. Camel exports also contribute to the foreign currency earnings of the country. However, since camels are used almost exclusively in subsistence production, the contribution of their products to the overall agricultural production is difficult to estimate.

Despite its high productive potential, camels perform poorly in the pastoral herd. Poor management, inadequate nutrients, slow production, and disease appear to be major constraints to the higher productivity of camels. Due to over-browsing shrubs and recurrent drought, camels graze and browse freely mixing with other livestock. As a result of this exposure of camels to contagious diseases and various endo- and ectoparasites, illnesses are expected to increase. Among the diseases that can possibly be cross transmitted between cattle, goat, sheep, and camel, is brucellosis.

The prevalence of brucellosis in camels (mainly in male camels) has been rarely studied. Therefore, the purpose of this study was to determine the seroprevalence of brucellosis in male camels.

MATERIALS AND METHODS

This study was carried out on 1500 apparently healthy male camels (*Camelus dromedarius*), which were intended for export. 1149 camels from Borena and 351 camels from Bale areas were brought to the Adama feedlot at different times during the period from December 2011 to April 2012. The animals were given numbers for the purpose of identification as soon as they reached the feedlot. They were fed on green grass, hay, and wheat barn and had free access to water. They were in good body condition. The animals were not vaccinated against brucellosis. They were allotted to three groups according to their ages based on the work of A b e b e et al. [3]. Group 1 (G1) consisted of camels 1 to 3 years old (n = 375), group 2 (G2) 3 to 6 years old (n = 937), and group 3 (G3), 6 years old and older (n = 188). A cross sectional study was designed. Blood samples were obtained by jugular venupuncture using plain vaccutainer test tubes from properly restrained animals and were stored in an ice box. Serum was separated from the clotted blood as soon as the samples were received in the National Veterinary Institute Immunology Laboratory, Ethiopia. The serum was stored at –20 °C until further processing took place. The prevalence of brucellosis was determined by Rose Bengal Plate Test (RBPT) [4] using *B. abortus* antigen (Institute Puravier 326, Rue de la Galera 34097 Montpellier Cedex 5, France) as a screening test and was confirmed by Complement Fixation Test (CFT) [14] using *B. Abortus* antigen S99 (CVL, New Haw Wey Bridge, and Surry KT 15 3NB, UK), control sera and complement (Bgvv, Berlin, Germany), and 2% sheep RBC, prepared by the National Veterinary Institute, Ethiopia, were used in the study.

Data were entered to Microsoft Excel Sheet, coded and analysed using the statistical package for social sciences (SPSS) version 15.0. Descriptive statistics, such as proportion (percentage), were used to summarize the data and calculate seroprevalence of brucellosis (positive by CFT). The prevalence was defined as the proportion of male camels positive for antibodies against brucellosis by Rose Bengal Plate and Complement Fixation Test to the total number of male camels tested, which was expressed as a percentage.

RESULTS

All 1500 serum samples were initially screened by Rose Bengal Plate Test. The RBPT was read as positive with any degree of agglutination and negative when agglutination was absent. Of all RBP tested samples, 11 male camels were found brucellosis seropositive caused by *Brucella abortus* giving an overall prevalence of 0.7 %. The seropositive prevalence of brucellosis in the Bale area was 0.6 % (n = 2) and 0.8 % (n = 9) in the Borena area (Table 1). This showed that there was no significant difference in the prevalence of brucellosis between male camels brought from Borena and those from the Bale area ($X^2 = 0.168$; df = 1; p = 0.682).

Table 1. Prevalence of male camel brucellosis by origin as screened by RBPT

Origin	No. of camels tested	No. of positive camels	Prevalence
Bale	351	2	0.6
Borena	1149	9	0.8
Total	**1500**	**11**	**0.7**

It was also found that there was no significant difference in the prevalence of brucellosis between different age groups of male camels ($X^2 = 0.130$; df = 2; p = 0.937). However, the highest prevalence was in the young age group (G1) (0.8 %) and the lowest in fully grown adult camels (G3) (0.5 %) (Table 2).

Table 2. Prevalence of camel brucellosis among different age groups as screened by RBPT

Origin [years]	No. of camels tested	No. of positive camels	Prevalence [%]
G1 (1—3)	375	3	0.8
G2 (3—6)	937	7	0.7
G3 (>6)	188	1	0.5
Total	**1500**	**11**	**0.7**

RBPT screened seropositive samples were subjected to Complement Fixation Test (CFT) for further confirmation.

All RBPT brucellosis seropositive male camels from Bale area were also found to be seropositive by CFT, whereas of the 9 (0.8 %) RBPT brucellosis seropositive male camels from Borena area, only 6 (0.5 %) were confirmed to be brucellosis seropositive by CFT (Table 3).

Table 3. Seroprevalence of male camel brucellosis according to different tests

Origin	No. of camels	RBPT		CFT	
		Positive	%	Positive	%
Bale	351	2	0.6	2	0.6
Borena	1149	9	0.8	6	0.5
Total	**1500**	**11**	**0.7**	**8**	**0.5**

In this study, the confirmatory test by CFT did not show significant difference in the prevalence of camel brucellosis in Bale and Borena areas ($X^2 = 0.011$; df = 1; p = 0.915). However, the prevalence was relatively higher in Bale camels (0.6 %) compared to those from the Borena area (0.5 %) (Table 4).

Table 4. Prevalence of male camel brucellosis from Bale and Borena areas as confirmed by CFT

Origin	No. of camels tested	No. of positive camels	Prevalence [%]
Bale	351	2	0.6
Borena	1149	6	0.5
Total	**1500**	**8**	**0.5**

No significant difference in the prevalence of camel brucellosis in different age groups was found ($X^2 = 0.00$; df = 2; p = 1.00) (Table 5).

Table 5. Prevalence of male camel brucellosis by age as confirmed by CFT

Age [year]	No. of camels tested	No. of positive camels	Prevalence [%]
G1 (1—3)	375	2	0.5
G2 (3—6)	937	5	0.5
G3 (>6)	188	1	0.5

DISCUSSION

Brucellosis remains one of the most common zoonotic diseases worldwide with more than 500,000 human cases reported annually [11]. The bacterial agent of brucellosis is classified by the CDC [7] as a category (B) pathogen that has a potential for the development as a bio-weapon. Camels are highly susceptible to brucellosis caused by *Brucella abortus* and *Brucella melitensis*. Brucellosis is transmitted from animals to humans by ingestion of raw milk, milk products, contaminated meat, raw liver, and close contact with animals through breeding, birth, slaughtering and inhalation of contaminated dust [8]. In the pastoralist and agro-pastoralist life style, raw; camel, cow, goat, and sheep milk is consumed. Fresh and non-boiled milk is considered best while still warm, as boiling is reputed to remove its "goodness".

The transmission of brucellosis to humans through infected and/or contaminated camel meat, by contact with infected carcasses, and through slaughtering is not usually considered by those directly involved. That is why there is little if any study conducted on the prevalence of brucellosis in male camels. Pastoralists sell male camels for slaughtering. They keep female camels for breeding and milk production, which is their staple food. Today in Ethiopia, not only pastoralists and agro-pastoralists, but also the urban populations in the pastoralist areas, consume camel's milk and meat. The seroprevalence of brucellosis in camels appears to follow two distinct patterns: low (2—5 %) prevalence in nomadic or extensively kept camels and high (8—15 %) prevalence in camels kept intensively or semi-intensively [1]. Teshome et al. [21] recorded 1.2 % seroprevalence of brucellosis in the Borena zone, 5.2 % in Afar and 2.8 % in Somalia regions of Ethiopia; 1.6 % in and around Dire Dawa city by Omer et al. [15], 1.8 % in the Borena zone by Megersa et al. [12], 6.95 % in the Sudan by Yagoub et al. [22], and 1.9 % in Somalia by Baumann and Zessin [6]. In this study, the low prevalence of *Brucella* infection (0.5 %) was observed because the study was carried out only on male camels. The incidence of brucellosis is significantly less in male than in female camels [12]. The other reason for the low prevalence of brucellosis in this study was probably because both the RBPT and CFT were conducted only against *Brucella abortus* while camels are also affected by *Brucella melitensis*. As the study camels were reared by extensive farming systems, the low seroprevalence of brucellosis in this study is in agreement with the findings of Abbas and Agab [1].

The seroprevalence of brucellosis in male camels from the Bale area (0.6 %) was relatively higher than in male camels from Borena area (0.5 %; p = 0.915) as confirmed by CFT. This is probably due to the fact that the Bale lowland area was affected by recurrent drought and pastoralists migrated with their camels to higher land areas where pastures are available and were obliged to make their camels graze/browse with cattle, sheep, and goat. Brucellosis can cross-transmit between cattle, sheep, goat, and camel [10]. Megersa et al. [12] pointed out that contact between camels and ruminants were more incriminated for the transmission of brucellosis to the camels. Abdel Rashed and Abdel [2] in their

study also reported a 9.19 % seroprevalence of brucellosis in camels in close contact with farm animals comparing to 8.68 % in slaughtered non-contact animals.

In this study, there was no difference in the seroprevalence of brucellosis among different age groups (0.5 % for all age groups). Similar result was obtained by Radwan et al. [16]. This might be associated with the management system of camel where both age groups were kept together in housing, pasture and watering.

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Received January 13, 2014

FOLIA VETERINARIA, 58, 1: 9—13, 2014

MAGGOT DEBRIDEMENT THERAPY FOR SUCCESSFUL HORSE WOUND TREATMENT (A CASE REPORT)

Gajdošík, M.[1, 2], Kozánek, M., Jr.[2], Takáč, P.[2, 3]

[1]St. Michael's Hospital, Department of Surgery, Cestana Červený most 1, Bratislava
[2]Scientica s. r. o., Hybešova 33, 831 06 Bratislava
[3]Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, 84506 Bratislava
The Slovak Republic

peter.takac@savba.sk

ABSTRACT

Maggot-therapy is the application of sterile fly larvae to chronic wounds to debride the wound bed of necrotic tissue, reduce bacterial contamination and enhance the formation of healthy granulation tissue. Maggot-therapy is used relatively little in veterinary medicine. In our study, we aimed to prove that this therapy deserves further consideration in veterinary medicine in the fight against aggressive and otherwise uncontrollable infections. The object of our study was a 21year old English thoroughbred stallion. At the end of January 2013, in an accident, he caused an incision wound on his left hind leg. After completing maggot debridement therapy, the wound healed completely and currently the horse leg is absolutely healthy and fully functional.

Key words: blowfly; larva therapy; maggots; veterinary medicine; wound management

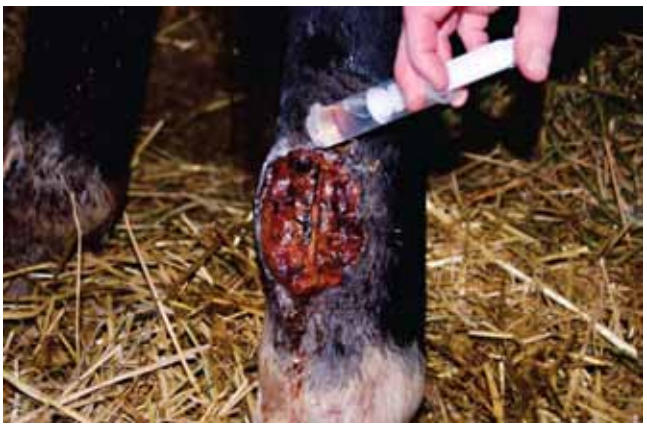
INTRODUCTION

The growing interest in maggot-therapy in human medicine suggests that it may also be of value to veterinarians. Maggot-therapy involves the application of disinfected fly larvae to chronic wounds to debride the wound bed of necrotic tissue, reduce bacterial contamination of the wound and enhance the formation of healthy granulation tissue. It is known by several names, including biodebridement, larval therapy and maggot debridement therapy (MDT). The use of maggot-therapy in human medicine has been practised sporadically for centuries [13], [14]. Prior to the development of antibiotics, it was used particularly for the treatment of various types of necrotising wounds, including chronic leg ulcers and osteomyelitis and greatly improved the prognosis for patients with such conditions [1], [11]. With the advent of antibiotics, however, maggot-therapy fell out of favour, until the emergence of strains of bacteria with multiple antibiotic resistance [12], since when it has experienced a resurgence of interest [3], [10]. Maggot-therapy is, however, little known in veterinary medicine. Nevertheless, growing antibiotic resistance and the increase in demand for organic husbandry and residue-free meat and milk, suggest that it is an option which merits further consideration. Until recently, maggot debridement therapy (MDT) had been reported only rarely for the treatment of wounds in veterinary species [5], [4], [2], [16]. Between 1997 and 2003, one author's laboratory (R.A.S.) distributed medical grade maggots to eight veterinarians who were each surveyed to investigate their reasons for the choice of this treatment and to learn about their clinical experiences from using it [15]. The majority of treatments were administered to horses; our case report also describes the successful treatment of a horse wound.

MATERIALS AND METHODS

Sterile Maggot Lucilia sericata preparation and application

The Maggot Therapy Laboratory at the Biotherapeutical facility of SCIENTICA, Ltd., at St. Michael's Hospital, Bratislava, Slo-



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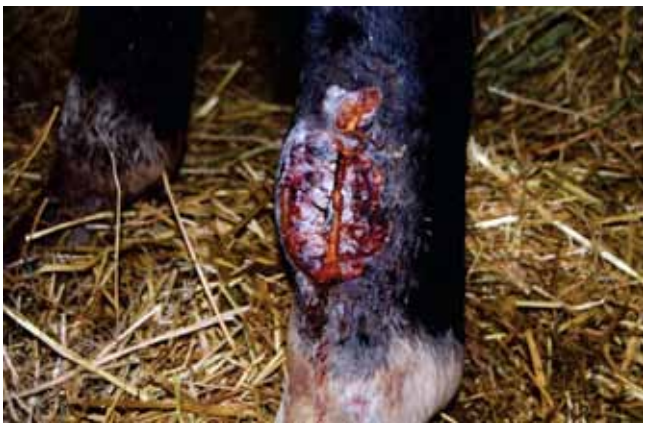

Fig. 2.

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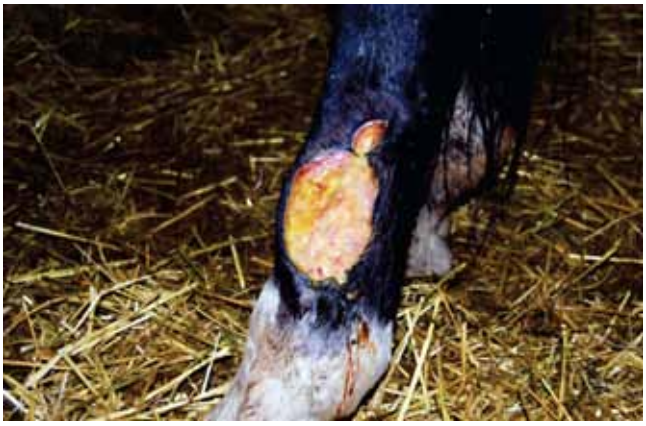

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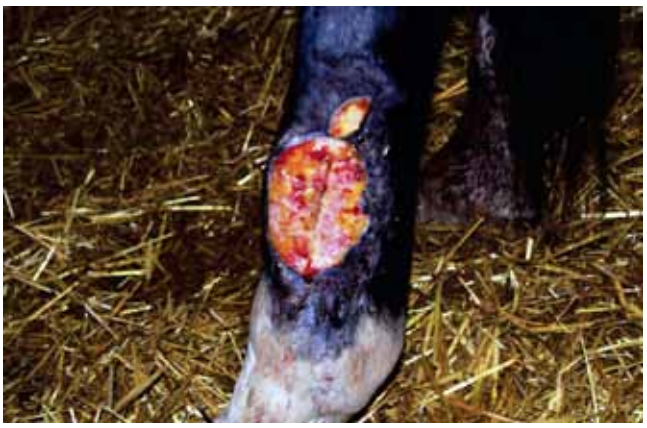
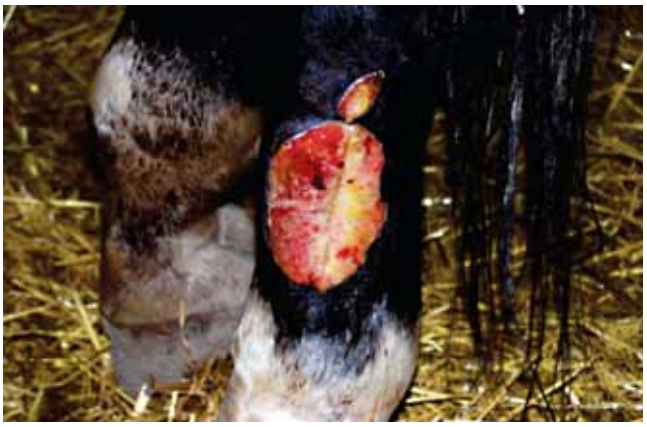


Fig. 9.

Fig. 10.

Fig.11.



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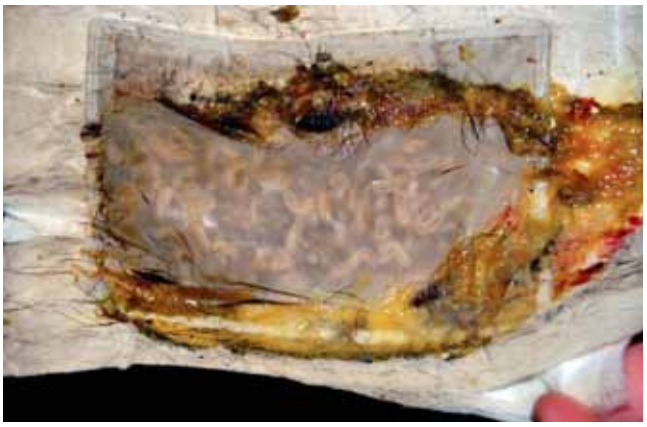

Fig. 14.

Fig. 15.

Fig. 16.

vakia, has made disinfected larvae (*Lucilia sericata*) available for clinical use when requested by physicians and veterinarians. For horse wound treatment, bags with sterile larvae (*L. sericata*) were provided free of charge to the owner of the horse participating in the study. Bags containing young second-instar larvae were prepared individually for each patient to match the size of the wound based on the physician's instructions. The dimensions of the bags ranged from 12 × 7 cm.The total number of maggots in the bag was 420 individuals, but always corresponded to 5 larvae.cm^{-2} of the folded bag surface area. This was the standard dosage of bagged larvae. Bagged larvae were supplied in sterile plastic containers and placed in a transportation box (AcuTemp; 23 cm wide × 20.5 cm long × 25 cm high) together with 6 cooling pads (ClimSel C 7N; Climator, Sweden), which were previously cooled to 5—6 °C. The bags were applied for approximately 48 or 72 hours, depending on the medical personnel's evaluation of the wound. The condition of the wound was carefully monitored during the treatment.

Horse history and clinical examination

The object of our study was a 21year-old English thoroughbred stallion. At the end of January 2013, in an accident, he caused an incision wound on his left hindleg. On the same day, the veterinarian repaired his wound and prescribed antibiotics. Two days after the operation, the stitches on the leg had ruptured and the wound was left open. At this stage, it was not possible tore-stitch the wound. On the advice of a veterinarian, the wound was cleaned and treated with antibiotic backfill. In spite of thorough care, the wound did not heal, and a scab had formed n the wound surface, under which pus had accumulated. In the horse wound, necrotic tissue had formed, and his general health condition was deteriorating rapidly (horse lost weight, refused to eat, was lethargic, etc.). The size of the wound was 12 × 7 cm and extended the entire length of the wound (Fig. 1, 2). Ischaemic wound surface was crusted and later occurred the crack throughout the depth.

RESULTS

Maggot application

After the failure of a two-week medical treatment, it was decided to use sterile maggots to complete debridement of the wound.

First batch of application

Day 1: the scab on the wound was manually removed and the pus from the wound was flushed with a large volume of sterile saline (Fig. 3, 4). After that the biobag with a dimension of 12 × 7 cm, with 420 sterile larvae (Scientica Ltd.), was placed into the wound. The wound was dressed with saline-soaked gauze and bandaged with a sterile elastic bandage (Fig. 5).

Day 2: about 13 hours after the initial application, the wound was checked; only the outer bandage was observed. The wound was flushed with a large volume of sterile saline (Fig. 6, 7).

Day 3: the gauze was removed and the wound examined; the condition of the maggots in the biobag was reviewed and documented. The pus in the wound was slightly cleaner and the area of necrotic tissue had decreased significantly. The same biobag with maggots was re-deployed to the wound; the top was covered again with saline-soaked gauze and bandaged with a sterile elastic bandage (Fig. 8, 9).

Day 4: the first round of maggot treatment was completed. The biobag with fully developed maggots was removed and discarded. The wound was apparently cleaner, pus had disappeared, healthy granulation tissue covered approximately 50 % of the wound surface, and the previously necrotic tracts had apparently shrunk. The wound remained a pink colour (Fig. 10, 11).

Day 5 to Day 7: the wound was simply wetted with sterile saline and covered with a sterile elastic bandage. The wound began to form a yellow pus coating again.

Second batch of application

Day 8: the wound was again flushed with a large volume of sterile saline. After that, the biobag (dimension 10 × 6 cm), containing 300 sterile larvae was placed into the wound. The wound was dressed with saline-soaked gauze and bandaged with a sterile elastic bandage.

Day 9: the wound was uncovered, checked and documented. The yellow coating on the wound was significantly reduced and had almost completely disappeared (Fig. 12).The same biobag with maggots was re-deployed on the wound, the top was covered again with saline-soaked gauze and the wound was bandaged with a sterile elastic bandage (Fig.13).

Day 10: the wound was checked and documented; only the outside bandage was assessed.

Day 11: the maggot debridement therapy was completed. The biobag was removed and discarded (Fig. 14). Necrotic tissue from the wound completely disappeared and the wound was clean and pink, without pus. Healthy granulation tissue covered approximately 80 % of the wound surface. The wound continued to contract. Overall, the wound size reduced to 9 × 6 cm (Fig. 15).

DISCUSSION

Maggot debridement therapy has previously been successfully used in a wounded bull [4], and in two donkeys [2]. US veterinarians who had employed MDT were surveyed to investigate their reasons for the choice of this treatment and their clinical experiences. Between 1997 and 2003, 13 horses were treated by eight veterinarians who used MDT to control infection or debride wounds which could not easily be reached surgically or were not responding to conventional therapy. Seven animals were lame, and six were expected to require euthanasia. Following maggot-therapy, all infections were eradicated or controlled, and only one horse had to be euthanised. No adverse events were attributed to maggot-therapy for any of these cases, other than presumed discomfort during therapy. The data collected suggest that maggot-therapy could be useful for treating some serious equine hoof and leg wounds [15].

Maggot-therapy is likely to carry the same potential complications as naturally-occurring myiasis: spread of infection

(especially if larvae are not disinfected), and tissue invasion (if invasive species or excessively high densities are used). Blowfly strike in sheep is associated with elevations of serum ammonia, hepatic encephalopathy, coma, and death [8, [9], [6]. The pathology associated with clinical blowfly strike is dependent upon high maggot load [8]. Nevertheless, therapists should be observant for these same signs during treatment with MDT, especially when using large quantities of larvae. The risk of invasive myiasis is likely to be one of the factors that have prevented veterinarians from considering maggot-therapy in the past. While therapeutic myiasis must be approached with caution, the experience of the veterinarians surveyed here indicates that MDT offers an acceptable level of safety with promising indications of efficacy in treating problematic equine wounds [15].

Maggot-therapy surely deserves further consideration in veterinary medicine in the fight against aggressive and otherwise uncontrollable infections [7].

CONCLUSION

The 21year-old English thoroughbred stallion horse showed no side effects, and there was no increase in body temperature. Throughout the maggot debridement treatment, the horse managed very well and was not nervous, which means that he did not perceive the larvae in the wound as a threat. During the period of treatment, the wound was moistened with sterile saline three times a day.

After completing maggot debridement therapy, the wound was still assessed for about six weeks with after care using colloidal silver and bandages.

After completing maggot debridement therapy, the wound healed completely and the horse leg is currently completely healthy and fully functional (Fig. 16).

Maggot debridement therapy can help in treating problematic equine wounds. Maggot-therapy was associated with complete wound healing in several injured animals that had failed to heal following conventional medical and surgical therapy. These results support the need for controlled clinical studies in order to define the indications and contraindications of MDT in equine wound management.

ACKNOWLEDGEMENTS

This study was funded by the Operational Program of Research and Development and co-financed by the European Fund for Regional Development (EFRD). Grant: ITMS 26240220020: Establishment of biotherapeutic facility and technology proposal for production and development of bio-drugs.

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Received January 20, 2014

FOLIA VETERINARIA, 58, 1: 15—20, 2014

STRUCTURAL AND ULTRASTRUCTURAL STUDY OF OVAL CELLS IN RABBIT'S LIVER AFTER BENDIOCARB ADMINISTRATION

Holovská, K.[1], Almášiová, V.[1], Cigánková, V.[1], Petrovová, E.[2]

[1]Institute of Histology and Embryology
[2]Institute of Anatomy
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

kholovska@uvlf.sk

ABSTRACT

Oval cells (OCs), or hepatic progenitor cells (HPCs) which originate from the cells present in the canals of Hering, are thought to be the progeny of stem cells in the adult liver. They are bipotent cells that are able to differentiate into either hepatocytes or cholangiocytes. In all specimens studied, oval cells have been observed. In humans, these cells have been called progenitor cells, while in rodents, they are referred to as oval cells. These cells do not represent a single cell type, but are a heterogeneous population of immature hepatic cells. The basic knowledge about the size, location, and morphology of these liver stem cells is still lacking. These cells are activated when the mature hepatocytes are continuously damaged or inhibited in their proliferation. They expand from the periportal areas to the pericentral zones and give rise to hepatocytes or cholangiocytes.

In this study, we identified OCs by light and electron microscopy in the rabbit liver after bendiocarb administration. They were found in the periportal areas as well as in the liver lobules between hepatocytes. OCs are closely associated with the inflammatory processes. The number of OCs depend on the intensity of inflammation. All of these cells have common characteristics. The OCs had an oval nucleus with heterochromatin and a cytoplasm which contained a moderate number of organelles.

Key words: bendiocarb; liver; oval cells; rabbit; ultrastructure

INTRODUCTION

Carbamate insecticides are widely used in homes, gardens and agriculture. They cause reversible carbamylation of the acetylcholine esterase enzyme (AChE), allowing accumulation of acetylcholine, a neuromediator substance, at parasympathetic neuroeffector junctions (muscarinic effects), at skeletal muscle myoneural junctions and autonomic ganglia (nicotinic effects), and in the brain (CNS effects). Symptoms of acute carbamate poisoning include weakness, blurred vision, headache, nausea, abdominal cramps, chest discomfort, myosis, sweating, muscle tremors and incoordination, decreased pulse, low blood pressure, heart irregularities, giddiness, confusion, slurred speech and loss of reflexes. Bendiocarb (2,2-dimethyl-1,3-benzodioxol-4-yl-N-methylcarbamate) is a broad spectrum insecticide belonging to the N-methyl carbamate group [2]. Bendiocarb can also affect some biochemical and haematological parameters [4]. Acute oral toxicity (LD_{50}) was investigated in various adult animals: rat 34—156 $mg.kg^{-1}$, guinea pig 35 $mg.kg^{-1}$, rabbit 35—40 $mg.kg^{-1}$ [35].

The liver is the major organ to manage the detoxification of exogenous and endogenous components and therefore is constantly exposed to different types of injurious agents. The repair of the liver is an intrinsic defence mechanism in order to protect the liver from injury. In the normal adult liver there is very little cell proliferation. Hepatocytes have a life span of over a year. The resting hepatocytes have the ability to re-enter the cell cycle rapidly after an injury.

However, if the proliferative capacity of hepatocytes is blocked by the effects of different medicines, toxins or acute viral diseases, the hepatic progenitor cells are activated [14], [22], [26]. In humans, these cells have been called progenitor cells, but in rodents, they are called oval cells. These cells were termed "oval" because of their characteristic morphology; ovoid nucleus and high nuclear to cytoplasmic ratio [3]. OCs are bipotent cells, which can fully differentiate into either hepatocytes or cholangiocytes [33]. They are located in the smallest and most peripheral branches of the biliary tree ductules and canals of Hering [28], [29]. After their initial activation, OCs proliferate, migrate, and differentiate into either hepatocytes or biliary cells [8].

The heterogeneity of OCs is well known [7]. OCs represent a dynamic cellular compartment that continuously changes morphology and phenotype in correlation with differentiation [9]. On the basis of morphological characteristics, De Vos and Desmet [6] classified oval cell into three types. OCs type I were the less-differentiated type. They were the oval-shaped with a centrally located oval nucleus, and with a small amount of organelles in their cytoplasm. OCs type II had the same general features as type I cells, however, they were more differentiated cells. The last type of OCs, type III, displayed features of hepatocytes including a prominent nucleus and voluminous cytoplasm. They were located in different regions of the hepatic lobules [6].

In the regions, where OCs are located, there is the specific microenvironment which supports them [20]. This is composed of numerous different cells such as hepatic stellate cells/myofibroblasts, endothelial cells, Kupffer cells, and cholangiocytes, which could interact with OCs. Moreover, inflammatory cells are responsible for producing a range of cytokines and chemokines that could influence the OCs [1].

The aim of our study was to complete the previous observations of the structural and ultrastructural changes in the rabbit liver after short and long-term administration of bendiocarb, focusing on the proliferation of OCs.

MATERIALS AND METHODS

Animals and diets

In the experiments we used adult hybrid Hyla (Oryctolagus cuniculus) rabbits (54 days old) with a mean body weight of 2500 g. The rabbits were clinically healthy, kept in a well ventilated environment, received a standard diet (O-10 NORM TYP, Slovakia) and water ad libitum. They were divided into five groups of six animals each (control, sacrificed after 3, 10, 20 and 30 days of drug administration). Rabbits in all experimental groups received bendiocarb (96 % Bendiocarb, Bayer, Germany) *per os* at a dose of 5 $mg.kg^{-1}$ per day [21]. Animals in the control group did not receive bendiocarb. The experimental and control animals were killed with thiopental (Thiopental Valeant 1 g, ICN, Czech Republic; 100 $mg.kg^{-1}$ b. w.) intravenously at days 3, 10, 20 and 30 after bendiocarb treatment [23]. This experimental study on rabbits was approved by the Ethic committee of the University of Veterinary Medicine and Pharmacy in Kosice (No. 2647/07-221/5).

Light microscopy (LM)

Histological samples of the liver for light microscopy were processed by a common histological technique. They were fixed in 4 % neutral formaldehyde and embedded in paraffin. Then 7—12 μm thick slides were stained with haematoxylin and eosin and photographed under a Jenamed light microscope.

Transmission electron microscopy (TEM)

The liver samples intended for transmission electron microscopy were fixed in 3 % glutaraldehyde, postfixed in 1 % OsO_4 (both in a phosphate buffer pH 7.2—7.4), dehydrated in acetone and embedded in Durcupan ACM (Fluka). The ultrathin sections were cut on a Tesla BS 490 ultramicrotome, stained with uranyl acetate and lead citrate and evaluated under a Tesla BS 500 transmission electron microscope.

RESULTS

Light microscopy

In the control animals, the liver displayed a normal histological structure. The polyhedral hepatocytes were arranged in anastomosing cords, separated by the liver sinusoids. Small amount of connective tissue was observed only in the portobiliary space. The portobiliary space contained an interlobular vein, artery and a biliary duct (Fig. 1a).

On day three of the experiment the most pronounced changes were observed in the portobiliar spaces which were edematous and contained inflammatory cells. In the portobiliary spaces OCs were observed. They occurred separately or in small groups containing three to five cells (Fig. 2). On the tenth day of the experiment, changes in the liver were not uniform. We observed moderate to slight inflammation in the portobiliary spaces. This was reflected in the number of oval cells which decreased considerably (Fig. 3). On the twentieth day of the experiment, the OCs exhibited morphological changes similar to those observed on day 10 (Fig. 4). On thirtieth day of the experiment, the OCs showed changes that differed between individual zones of liver parenchyma. We observed moderate hyperaemia. Some portobiliary spaces were free of inflammatory cells but in others, a slight inflammation was observed (Fig. 5). OCs were observed not in the portobiliary spaces, but in the liver lobuli (Fig. 6). In the experimental groups the number of OCs depended on the intensity of inflammation.

Electron microscopy

The control group hepatocytes had normal ultrastructure (Fig. 1b).

On day three of the experiment, we observed OCs located close to the periportal areas. OCs have oval nuclei with heterochromatin located at the periphery. The cytoplasm contained a moderate number of organelles, and the scattered mitochondria were round to oval. Some OCs were situated between hepatocytes or they penetrate in the perisinusoidal space and were wedge-shaped. Junctional complexes were well-developed between these cells and neighbouring hepatocytes. The desmosomes were clearly visible (Fig. 7).

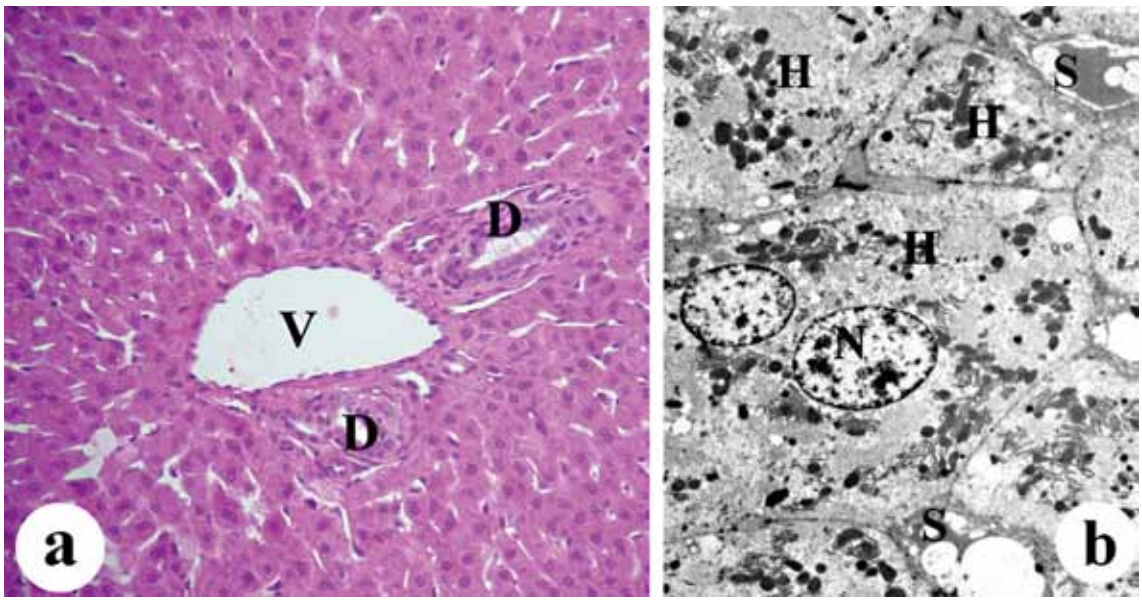

Fig. 1. Light (a) and electron (b) microscopy of liver from the control group
a: Hepatocytes were arranged in anastomozing cords; V — interlobular vein; D — interlobular bile duct. Magn. ×200
b: H — hepatocyte; N — nucleus; S — sinus. Magn. ×1 800

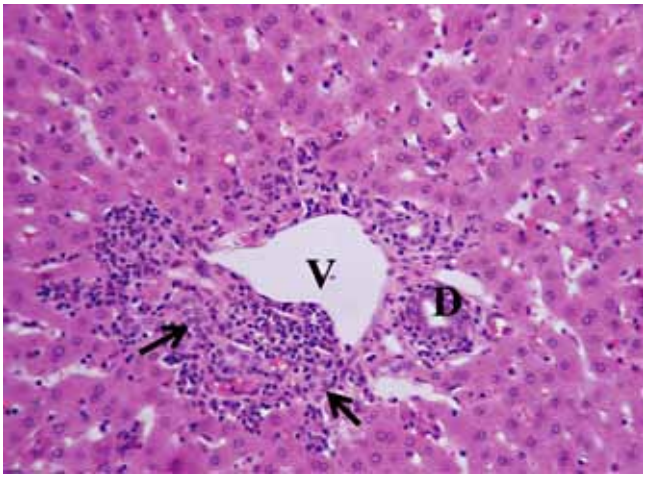

Fig. 2. Liver of a rabbit on day 3 of the experiment (H–E). Magn. ×200
D — interlobular bile duct; V — interlobular vein; arrow — oval cells

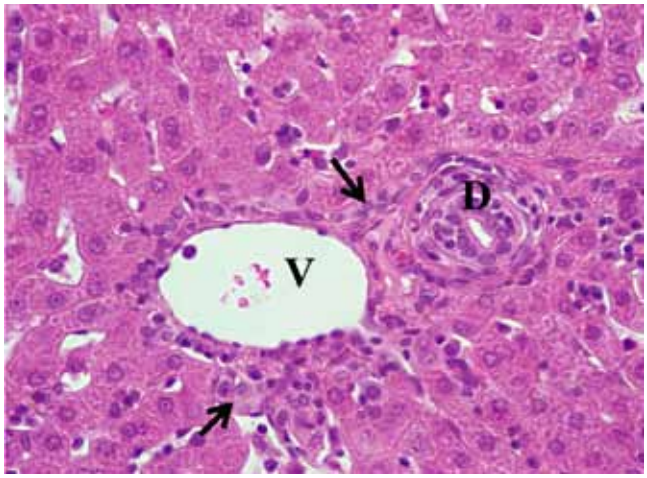

Fig. 3. Liver of a rabbit on day 10 of the experiment (H–E). Magn. ×400
D — interlobular bile duct; V — interlobular vein; arrow — oval cells

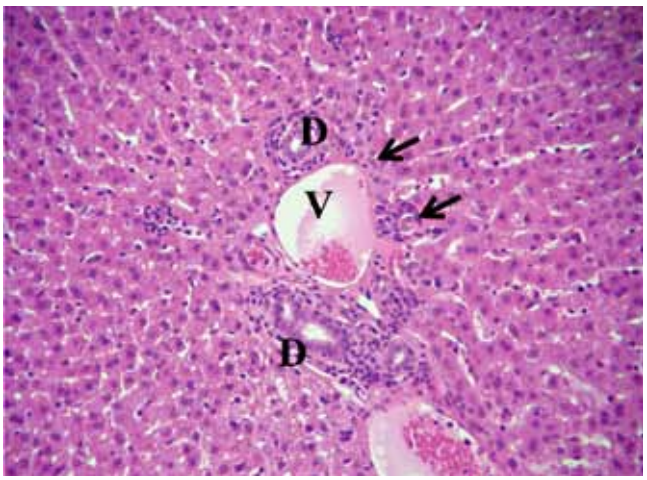

Fig. 4. Liver of a rabbit on day 20 of the experiment (H–E). Magn. ×200
D — interlobular bile duct; V — interlobular vein; arrow — oval cells

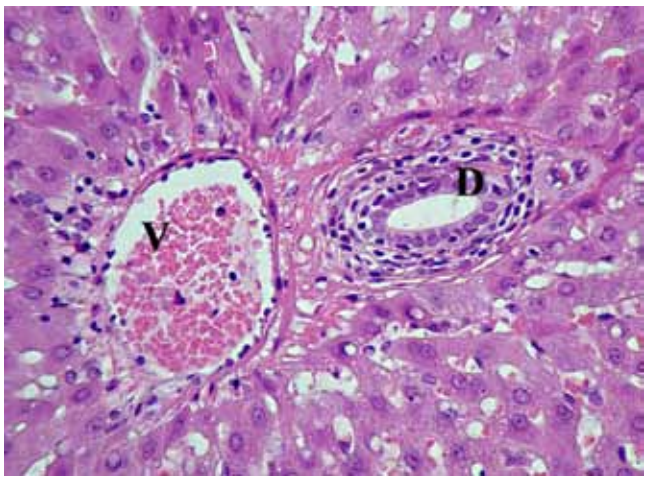

Fig. 5. Liver of a rabbit on day 30 of the experiment (H–E). Magn. ×400
D — interlobular bile duct; V — interlobular vein

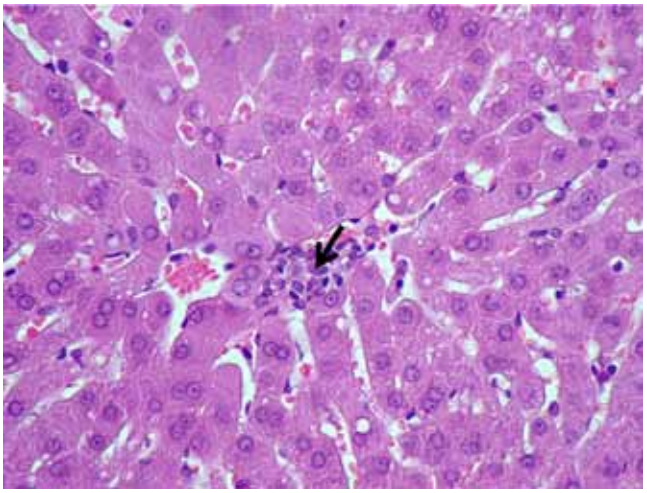

Fig. 6. Liver of a rabbit on day 30 of the experiment (H–E). Magn. ×400
arrow — oval cells

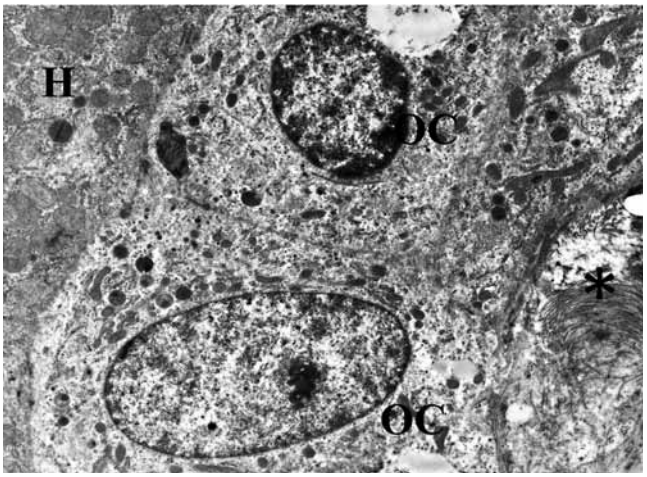

Fig. 8. Electron micrograph of a liver on day 10 of the experiment
Magn. ×4 320 . H — hepatocyte; OC — oval cell; asterisk — collagen fibres

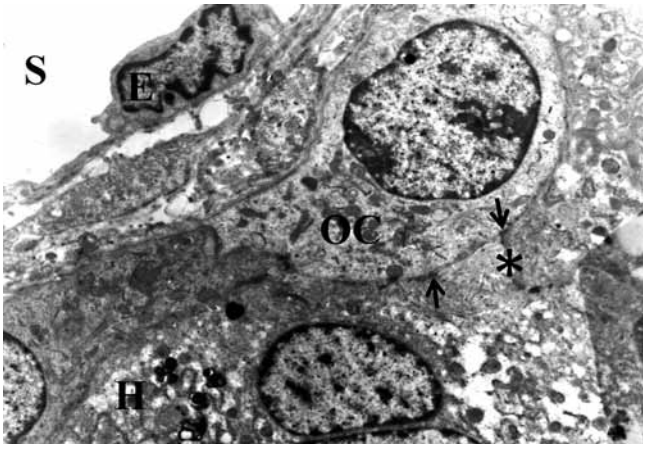

Fig. 10. Electron micrograph of a liver on day 30 of the experiment
Magn. ×4 200
H — hepatocyte; E — endothelial cell; S — sinusoid; OC — oval cell;
arrow — intercellular contact; asterisk — collagen fibres

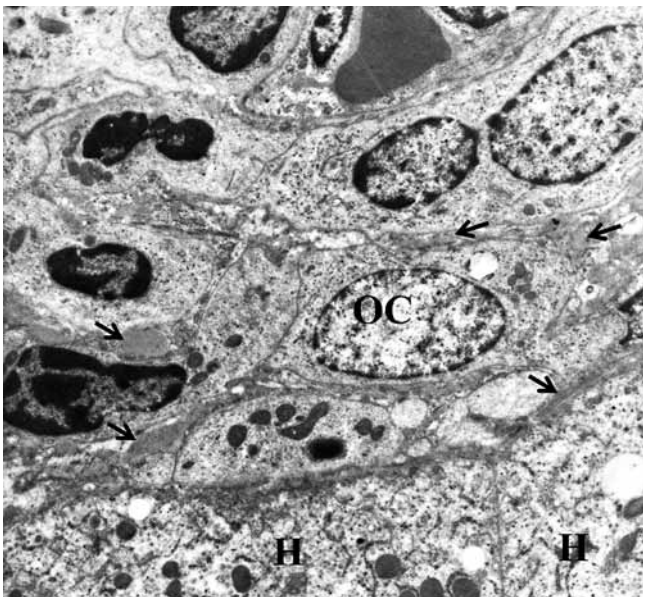

Fig. 7. Electron micrograph of a liver on day 3 of the experiment
Magn. ×2 700. H — hepatocyte; OC — oval cell; arrow — collagen fibres

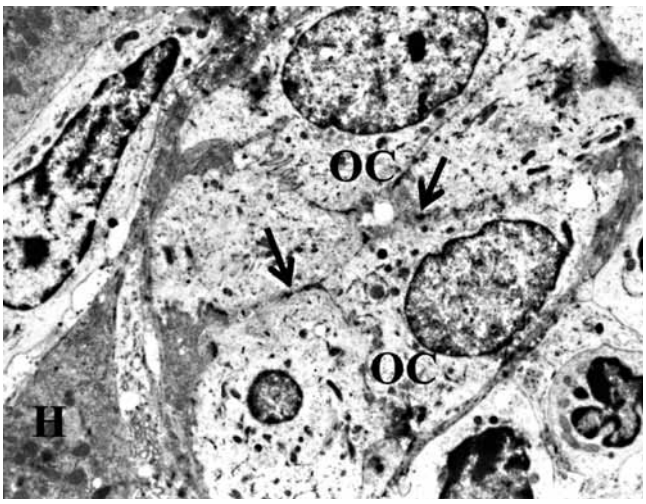

Fig. 9. Electron micrograph of a liver on day 20 of the experiment.
Magn. ×2 500
H — hepatocyte; OC — oval cells; arrow — intercellular contact.

On day 10 of the experiment, the OCs had the same general characteristics as OCs on day 3 of the experiment. However, they were larger and their cytoplasm contained more organelles. Rough endoplasmic reticulum was moderately developed, a few prominent lysosomes were visible, and between adjacent cells, the bile canaliculi were clearly visible (Fig 8).

On day 20 of the experiment, OCs had the similar morphological features as on day 10 (Fig 9).

On day 30 of the experiment, the OCs had a structure which resembled the mature hepatocytes. These cells were larger with prominent nuclei, and voluminous cytoplasm

with numerous organelles. These cells formed intercellular canaliculi with the neighbouring hepatocytes. Their cells membranes were bound together by intercellular contacts as desmosomes and tight junctions (Fig. 10).

DISCUSSION

It is known that the toxicity of bendiocarb is manifested through interaction and inhibition of acetylcholine esterase. Generally carbamates are excreted rapidly and do not accumulated in mammalian tissues. Although they are excreted, their toxic effect lies in the cumulative effect of all their metabolites. Our previous study demonstrated that bendiocarb caused a structural change in the rabbit liver [11].

The liver has enormous regenerative capacity. If hepatocytes proliferation is blocked, the OCs are activated. These cells represent the most important regenerative alternatives during conditions when tissue injury is too severe [19]. Numerous animal and human models of liver damage or diseases exist in which the proliferation of OCs has been documented [7], [13], [36]. We previously observed that OCs were located in close association with inflammatory cells [12]. Further studies by Libbrecht et al. [16], reported a correlation between the number of OCs and the degree of inflammatory infiltrate. The inflammatory cells may activate OCs *via* cytokine and growth factors [17]. Our ultrastructural observations revealed different types of OCs located in different regions of the hepatic lobules. On day 3 of the experiment, OCs were observed mainly in the portal regions. After their activation, they migrated into the hepatic lobules, where they presumably underwent further differentiation into mature hepatocytes.

The available literature indicate that non-parenchymal cells play the most important role in liver regeneration. A study on animal models by Van Hul et al. [34], indicated that OCs need a supported matrix, provided by hepatic stellate cells (myofibroblasts), for migration and anchorage. Hepatic stellate cells (HSC) are closely associated with OCs [22], [25], [34]. These cells take part in extracellular matrix synthesis and remodelling, which is very important to OCs proliferation [34]. Chen et al. [5] observed that in the early phase of liver regeneration, HSCs stimulated OCs by hepatocyte growth factor. Petrovová et al. [24] observed in the rabbits exposed to bendiocarb, an increase in the quantity of collagen fibres in perisinusoidal and pericellular spaces. Light microscopy revealed numerous OCs in the regions where the quantity of connective tissues was increased. On the day 3 of the experiment, OCs were observed in portobiliary spaces, and later within the liver lobules or at their periphery [12].

As mentioned above, OCs were observed in large numbers on day 3 of our experiment, the time of the highest inflammatory intensity in the periportal areas. They occurred separately or in small groups containing three to five cells. All cells had common characteristics. They were small in size with oval, euchromatic nuclei and they were bound to adjacent cells with junctional complexes. The cytoplasm contained a moderate number of organelles. Gradually, with decreasing intensity of inflammation, the number of OCs also decreased [12]. Later, on day 10 of our experiment, OCs were scattered throughout the parenchyma, where they gradually differentiate into hepatocytes. They showed almost the same features as hepatocytes.

According to recent information, the degree of OCs activation increases with the severity of tissue injury [10], [16], [17], [32]. A trigger for progenitor cell activation is certainly the lack of ability of mature hepatocytes to proliferate [30]. Rat models of impaired hepatocyte replication showed that oxidative stress plays an important role in the activation of liver progenitor cells [28]. Roskams et al. [28] demonstrated that reactive oxygen species (ROS) production by liver mitochondria is increased significantly in mice with diet-induced steatohepatitis. They assumed that increased H_2O_2 production by hepatocytes is the general mechanism for the inhibition of the replication of mature hepatocytes during chronic oxidative stress. This observation supports the concept that OCs expansion is a component of the liver's adaptive responses to oxidative stress [28].

Sobeková et al. [31] observed the increased synthesis of manganous superoxide dismutase (Mn SOD), on days 10 and 30 of their experiments. This process indicates increased production of reactive oxygen species in mitochondria and a protective role of Mn SOD. Significantly increased content of thiobarbituric acid reactive substances (TBARS), observed in rabbits after administration of bendiocarbamate [31], suggests exactly such a process. Several studies indicate that inflammatory cells are a source of ROS during acute and chronic inflammatory diseases [27], [18]. Knight et al. [15] observed in mice liver, that the inflammatory response occurred immediately before oval cells numbers began to expand in the liver. This suggests that the expansion of the OCs begin shortly following liver damage and inflammatory cell infiltration.

CONCLUSION

In our study, we observed the effects of bendiocarb on the ultrastructure of OCs. They were closely associated with inflammatory processes. The largest number of OCs was observed on the third day of the experiment in the portobiliary spaces. Later, with the decreasing intensity of inflammation in portobiliary spaces, the number of OCs decreased. These cells are capable of migration into the parenchyma, therefore OCs were observed not only in portobiliary spaces but also in the liver lobuli. They have common morphological features, depending on the state of their proliferation and differentiation.

ACKNOWLEDGEMENTS

This study was supported by VEGA project No. 1/0111/13 from the Slovak Ministry of Education.

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Received February 4, 2014

FOLIA VETERINARIA, 58, 1: 21—24, 2014

PLATELET-RICH PLASMA, BIOLAMP AND LASER IN SKIN GRAFT HEALING

Capík, I.[1], Országhová, A., Kremeň, J.[2]

[1]Clinic of Small Animals
[2]Department of the Environment, Veterinary Legislation and Economy
University of Veterinary Medicine and Pharmacy, Košice
The Slovak Republic

igor.capik@uvlf.sk

ABSTRACT

Ten Hilla breed rabbits with a mean body weight of 2.9 ± 0.3 kg were used in this study. Each rabbit had four full-thickness skin grafts with the dimensions of 2 × 3 cm, two in the cranial part of the back and two in the caudal part of the back. Skin grafts were fixed at the recipient sites using single interrupted sutures. Four groups of skin grafts were created: control group (16 grafts), biolamp group (8 grafts), laser group (8 grafts) and platelet-rich plasma group (8 grafts). Skin grafts in the control group healed without any treatment. In the platelet-rich plasma group, plasma activated using bovine thrombin was administered below the muscle layer of the recipient site. Skin grafts in the laser and biolamp groups were treated for ten minutes, 3-times daily, for three weeks. Out of the 16 skin grafts included in the control group, 4 (25 %) were healed successfully with a healing time of 10.25 ± 0.6 days (mean ± SEM). Out of the eight skin grafts in the plasma group, 5 healed successfully (62.5 %) in a significantly shorter healing time in comparison with the control group. The Chi square test confirmed the statistical significance between the laser group and the plasma group and between the control group and the plasma group. The biolamp treatment resulted in slowed down skin graft necrosis, comparable to the platelet rich plasma group as confirmed by the Anova test.

Key words: biolamp; laser; platelet rich plasma; rabbit; skin graft

INTRODUCTION

The surgical treatment of wounds has been focused primarily on accelerating the healing process. The aim of this acceleration is to overcome the initial risk associated with bleeding and infections. The principal differences in the wound healing process lie within the wound itself, specifically its depth, localization, aetiology and other associated pathologies [5].

Specific growth factors, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF), have been used in topical treatments but the results published thus far are inconclusive. This could be because of the complexity of the healing phenomenon, which cannot be stimulated by the local application of one factor at any given time, especially since the dosages are often well above physiological levels [2], [4].

Skin grafts are routinely used to close open skin defects that can't be primarily closed or to replace irreparably damaged skin. Restoration of the barrier and mechanical functions of the skin protects against infection, offers pain relief, decreases evaporative fluid and heat loss, restores thermoregulation, and renews metabolic and sensory functions. Skin grafts can be obtained from several sources for both humans and other animals. Skin grafts can include all or a portion of the skin including the epidermis and dermis. More recently, the use of skin substitutes, dermal fillers, and tissue expanders have increased surgeon's and dermatologist's ability to cover skin defects resulting from burns, traumatic injury, chronic wounds, or the excision of cancerous lesions.

Skin grafting is the removal of skin from one part of the body, the donor site, and its placement on another part of the body, the recipient site. Grafts of any kind must acquire vascularization from the recipient bed to survive [6], [12]. The major sources of skin grafts are autografts, allografts, and xenografts. Various skin substitutes were developed, including epidermal, dermal, or composite materials using human and animal or engineered materials. Autologous skin grafts in small animal practice are mostly used in acute or chronic wounds with loss of the skin at the distal leg regions. One of the theories has been that haemoglobin level should be maintained above 10 g.dl^{-1} to promote wound healing [7]. Agarwal et al. [1] concluded that prophylactic transfusions to increase the oxygen carrying capacity of the blood for the purpose of wound healing is not indicated in asymptomatic normovolaemic anaemic patients (with haemoglobin levels greater than 6 g.dl^{-1}) without significant cardiovascular or pulmonary disease.

This study was conducted with the aim to clinically compare the influence of platelet rich plasma, laser and biolamp therapies at revascularization of skin grafts in rabbits.

MATERIALS AND METHODS

The study was conducted on 10 rabbits of the Hilla breed weighing 2.9 ± 0.3 kg. Full-thickness skin grafts were collected using a scalpel from the lumbar area and dorsal thoracic region post-scapular bilaterally. Each of twelve rabbits had four skin grafts with the dimensions of 2 × 3 cm. Lumbar skin grafts were removed and replaced in the post-scapular area and post-scapular skin grafts were relocated in the lumbar area. This procedure was performed by two surgeons, resulting in immediate relocation of the created full skin grafts with minimal chance for loss of tissue liquids from the graft and drying of the recipient bed. After changing, skin grafts were fixed at the recipient site using single interrupted sutures (nylon monofilament EP 2 — Resolon) around the perimeter and minimally, two sutures in the middle of skin graft to preclude accumulation of wound exudate between the graft and the recipient bed. We have created the following four groups of skin grafts:

1) Control group without treatment,
2) Group treated using biolamp Bioptron for 10 minutes, 3 times daily for three weeks.
   The Bioptron lamp is a light source emitting polychromatic light with the wavelength of 480—3400 nm. Light has a high degree of polarization (more than 95 %) and low energy 2.4 J.cm^{-2}.
3) Group treated by laser BTL 2000. BTL-2000 is a portable laser therapy, which uses red and infrared laser probes. Parameters used in therapy: dose (J.cm^{-2}) — the amount of energy emitted into the treatment area — 48 J.cm^{-2}, the frequency of 6 Hz, 100 mW, exposure time 10 minutes at a distance 5 cm, 3 times a day for three weeks.
4) Group treated by administration of platelet rich plasma (PRP), activated by bovine thrombin, into the muscle layer below the skin graft during surgery.
   PRP was prepared using a two step procedure. The 15 ml of blood collected from *arteria cephalica antebrachii* was mixed with 1.5 ml sodium citrate. The blood was divided into three 5 ml centrifuge tubes and centrifuged at 1600 rpm (400 g) for 10 minutes. The lower half of the plasma together with the superficial layer of red blood cells was transferred to another test-tube and centrifuged at 2 500 rpm (900 g) for another 10 minutes. The plasma was removed within half a millilitre of the supernatant and after re-suspending the platelets in the plasma by gentle agitation, we obtained 2.5 ml of PRP.

The quality of the wound treatment was assessed by clinical examination and regular photographic documentation for three weeks. For statistical analysis, the Chi square test, *t*-test and Anova test were used to compare the time of healing period as the clinical sign of skin graft necrosis.

The Chi square test was used for statistical analysis for the whole contingency table with the result of dependence of therapeutic success and non-success, respectively. $P < 0.05$ was considered significant. Consequently, we performed the analysis between treatment methods. Further analyzes were carried out using *t*-test and ANOVA between the number of days that skin grafts clinically necrotized.

RESULTS AND DISCUSION

Ten rabbits were included in the study, each of them having four full-thickness skin grafts. Since all wounds were surgical in origin and their size did not differ, healing conditions were approximately the same. The only factor that could negatively affect revascularization of skin grafts in all of the groups of rabbits was the impossibility to eliminate shearing forces over the graft site.

Out of 16 skin grafts included in the control group, 4 (25 %) were healed successfully with a healing time of 10.25 ± 0.6 days (mean ± SEM).

Eight skin grafts treated with the laser succumbed to necrosis shortly after implantation. No graft treated by this method did retain vitality. The grafts acquired blue-black to black colour in a short time. Already 72 hours after implantation, these grafts were markedly dry (Fig.1).

In the biolamp group one skin graft healed by primary intention in ten days and seven of them necrotized. The healing time did not differ from the control group. Those defects healed by secondary intention below the dry gangrenous skin grafts without complications in 21—23 days.

The plasma rich group showed a significantly shorter healing time of 4.8 ± 0.4 days ($P < 0.05$) in comparison with the control group. On the sixth day, plasma rich grafts healed completely, while in the control and biolamp group crusts with minor exudation were still present around the perimeter of the grafts (Fig.1). Skin grafts in these groups were healed by the ninth to twelfth day (Table1).

Out of eight skin grafts in the plasma group, 5 healed successfully (62.5 %) resulting in shorter healing times in comparison with the control group. The chi square test confirmed the statistical significance between the laser and plasma groups and between the control group and the plasma group.

We found differences in the time of necrosis of skin grafts between groups. The laser group succumbed the fastest to necrosis of the skin grafts, i. e. after three days, followed by the control group, the biolamp group and finally the plasma group. Skin grafts in the laser group showed apparent loss of fluids already on the third day after implantation. On the sixth day, we observed marked graft contraction and separation of peripheral necrotic parts of the grafts (Fig. 1)

When comparing the number of days leading to the necrosis of the skin grafts, statistical significance had been confirmed between the control group and all other groups ($P < 0.001$), between the biolamp and laser groups, and the laser and plasma groups. Statistical significance had not been established between the biolamp and the plasma groups.

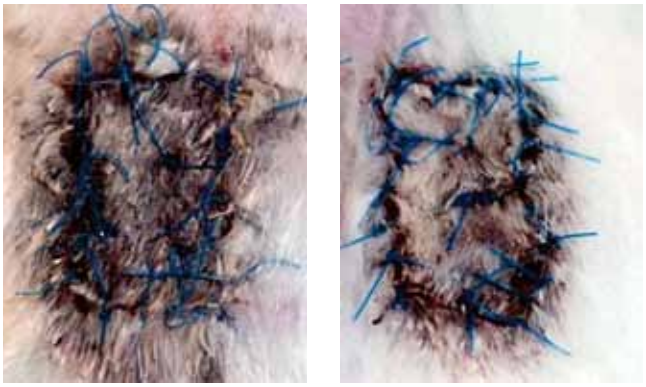
Table 1. Comparison of skin graft survival, period of necrobiosis and healing time among the groups

Treatment group	Number of skin grafts	Skin graft survival	Graft necrosis [day/number of grafts]
Control	16	4 (25 %)	4/8 5/4
Laser	8	0 (0 %)	3/8
Biolamp	8	1 (12.5 %)	4/3 6/4
Plasma	8	5 (62.5 %)	6/3

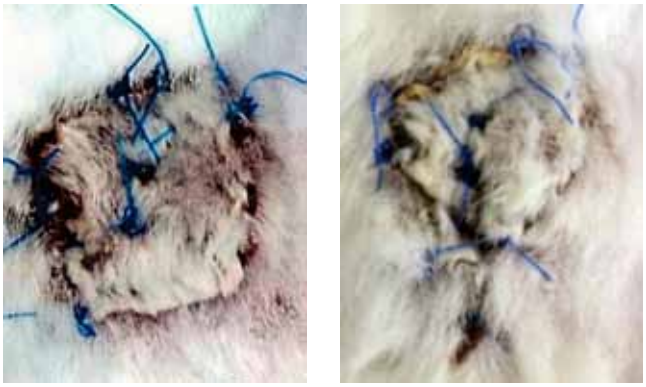
The use of PRP results in a greater similarity to the natural healing process, with the application of multiple growth factors in their biologically determined ratios, more closely than the addition of a single growth factor such as recombinant human platelet-derived growth factor-BB isomer (rhPDGF-BB) [2].

PRP has been found to be effective in several case control studies in human medicine [8], [11]. Clinical wounds are naturally highly variable, which may alter the results. The main differences are related to the wounds themselves (depth, localization, aetiology, and other pathology). This was the reason why we evaluated autogenous full-thickness skin grafts transferred to each other immediately after their excision. Skin grafts formed in this way practically did not differ among themselves in relation to the aetiology, localization, age and size of the wound, thus we tried to exclude the impact of these diverse factors on the healing process. Full-thickness skin grafts involving both layers of skin are mostly indicated for more severe injuries. These grafts are more complicated than partial-thickness grafts, but provide better contour, more natural colour, and less contraction at the grafted sites [9], [10].

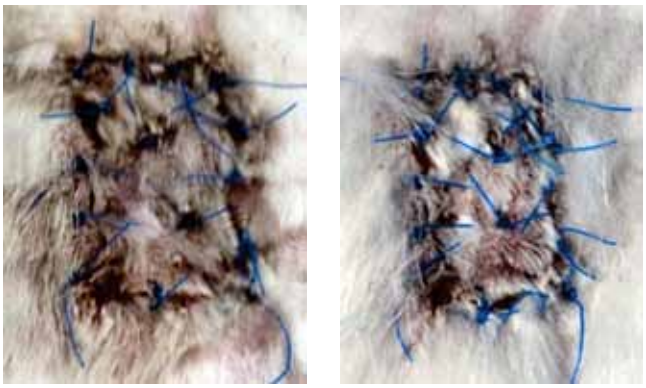
Group 1: Control



Group 2: Laser therapy



Group 3: Biolamp therapy



Group 4: Plasma therapy

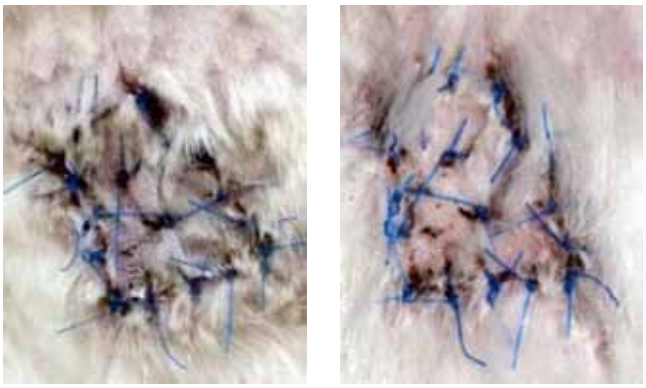


Fig. 1. Clinical picture of skin grafts 3 (left column) and 6 (right column) days following implantation

Our results confirmed the beneficial effect of platelet rich plasma on wound healing in comparison with the control group and the other tested groups. In the case of laser therapy, the therapeutic results pointed to a negative impact in relation to the time of the survival of skin grafts following implantation, as well as the overall success of healing by primary intention. None of the skin grafts in this group healed by primary intention. Our results also confirmed the positive influence of the wound rest during the healing process, seen in the case of the control group. Two rabbits with four skin grafts included in the control group, showed a 50 % success rate in healing, confirming the impact of the rest on the success of the graft revascularization. The importance of platelet-rich plasma in the healing of skin grafts compared to the control group was confirmed by the therapy success rate despite manipulation three times a day.

The risk of wound infection is directly related to the healing time [3], [13]. Additionally, wound infections increase exudation and pain leading to patient discomfort and an increase in the cost of the treatment. No infectious complications were observed in the groups. Rejected skin grafts healed secondarily during 20—23 days regardless of the method of therapy.

CONCLUSION

The results of this investigation showed that platelet rich plasma significantly promoted skin graft revascularization and reduced the healing time. The cost of autologous platelet rich plasma preparation is not high, which predisposes it for common use. Laser treatment had no beneficial effect on the healing of the skin grafts, while biolamp showed some effect on the healing process.

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Received, February 11, 2014

FOLIA VETERINARIA, 58, 1: 25—29, 2014

EFFECT OF PULSED ELECTROMAGNETIC RADIATION ON THE RAT TESTIS AND EPIDIDYMIS: HISTOLOGICAL STUDY

Almášiová, V.[1], Holovská, K.[1], Cigánková, V.[1]
Tarabová, L.[1], Beňová K.[2], Račeková, E.[3]

[1]Department of Anatomy, Histology and Physiology
[2]Department of Genetics and Biology
University of Veterinary Medicine and Pharmacy, Košice
[3]Institute of Neurobiology, Slovak Academy of Sciences, Košice
The Slovak Republic

viera.almasiova@uvlf.sk

ABSTRACT

The exposure of living organisms to electromagnetic radiation (EMR) is not a new phenomenon, but in the past few decades, environmental exposures to man-made EMR has been rapidly increasing and practically everyone has been exposed to the dense mixtures of electromagnetic waves without careful considerations of the possible health consequences. Wireless devices, such as: mobile phones, laptop computers and others, are very often worn and/or kept close to the reproductive organs either in standby position or during actual use. The histopathological study presented here was designed to determine the possible adverse effects of immediate, whole body EMR on the rat testis and epididymis. Sexually mature Wistar rats were irradiated by a pulsed electromagnetic field at a frequency of 2.45 GHz and a mean power density of 2.8 mW.cm^{-2} 3 h a day, for 3 weeks. Directly after the last exposure, the animals were euthanized and tissue samples for histological observations were obtained and processed by common histological techniques.

The testes in the experimental animals had considerably dilated blood vessels within the *tunica albuginea*, as well as, the testicular interstitium, and the testicular parenchyma consisted of irregular seminiferous tubules which were surrounded by enlarged interstitial spaces. The seminiferous epithelium contained empty spaces and the tubular lumina contained different amounts of immature sloughed spermatogenic cells. The Leydig cells did not show any obvious structural changes. The lumina of the *ductuli efferentes* were often filled with the clumps of immature spermatogenic cells and the ductus epididymidis contained masses of spermatozoa. The results of our study revealed the distinct adverse effects of non-ionizing radiation in the microwave range, on the histological structure of the rat testis.

Key words: adult rats; male reproductive system; microwaves; histopathology

INTRODUCTION

Electromagnetic fields are produced by the distribution and use of: electricity (low frequency), mobile phones, and other wireless technologies (high frequency, radiofrequency and microwaves). The use of modern advances in technology will continue to grow as providers proceed to offer more expansive services and improved products. Despite the number of benefits offered by these devices, we cannot overlook the fact that they create relatively dense networks of electromagnetic waves. However, despite the fact that the energy of the these types of waves are considered to be low powered, the chronic exposure may be as deleterious as the high level acute exposure [2], and this may present potential health risks. In general, according to the characteristics of the emitted waves, the exposure to EMR is divided into two categories: continuous and pulsed. The biological effects of pulsed waves are even more harmful than that of continuous [9]. The high frequency radiation — radio frequency, and microwaves, penetrate the tissues and the exposed molecules are forced to move about more rapidly and collide

with one another, causing increased friction and heating. This is considered as the thermal effect [26]. The non-thermal direct effect comprising a wide spectrum of different metabolic pathways is still under investigation. The subcellular mechanisms, include changes in plasma membrane potential with subsequent calcium efflux and alteration in many enzymes, ion pumps and proteins. The enhancement of apoptosis, reactive oxygen species (ROS) and heat shock protein production is also connected with EMR [4], [5], [15], [16], [23]. Such biological effects may or may not be reversible, depending on the type of irradiated tissue, intensity, frequency and duration of the irradiation.

The reproductive system, together with the central nervous system [21] and eyes [3], [32], are extremely sensitive to the EMR, because these structures are enveloped by a thick protective layer of dense connective tissue and therefore the ability to dissipate the heat from radiation is restricted. Heat generation depends on the specific absorption rate (SAR) and energy level — power density of emitted EMR, which must exceed 100 mW.cm^{-2} in order to cause the heating effect in the tissues [9]. In general, the testis represents a highly sensitive unit that requires the integration of intrinsic and extrinsic factors for its optimal function. The generated electrical current may alter the optimal testicular microenvironment and the hormonal status, both needful for successful spermatogenesis; therefore the testis and male reproductive functions are frequently included in EMR studies.

The objective of this study was to investigate the effect of immediate whole body irradiation by pulsed EMR on the histological structure of testes and epididymes in rats. The frequency of EMR of 2.45 GHz was selected because it is generated from the common human-made sources such the mobile phones or microwave ovens and the SAR was similar to the amount of daily exposure for the general public, and does not raise body temperature.

MATERIALS AND METHODS

Experimental design and animal handling

The experiment was carried out on sexually mature male rats, strain Wistar (n = 40) which were randomly allocated into two groups, control (n = 20) and experimental (n = 20). The clinically healthy rats were kept in ordinary cages (two rats per cage) at a controlled temperature of 21 ± 1 °C, and had ad libitum access to water and food (Larsen diet). The light was turned off or on using a 12 h regimen. The experimental rats were irradiated in a purpose-designed chamber by a pulse-wave EMF of 2.45 GHz, at a mean power density of 2.8 mW.cm^{-2} for 3 hours a day, during 3 weeks. Uniformity of the electromagnetic fields was analysed with a spectral analyser to determine the optimal placement of the animals [20]. Immediately after the last irradiation of the experimental rats, the animals from the experimental and control groups (non-irradiated) were anesthetized by i. p. injection of xylazine and ketamine, sacrificed by decapitation and their testes were sampled for histopathological evaluations. The control group rats were handled the same in every way as the experimental rats, with the exception that they did not receive any EMR.

The experiments were conducted at the Institute of Neurobiology, Slovak Academy of Sciences. The care and use of the animals were approved by the Ethics Committee of the Institute of Neurobiology, Slovak Academy of Sciences and the State Veterinary and Food Administration of the Slovak Republic.

Light microscopy

The tissue samples were immediately fixed in Bouin solution for 24 hours, then dehydrated and finally embedded in paraffin. Excisions were serially sectioned in 5 µm thick sections and stained with haematoxylin and eosin (H & E). Tissue sections were examined by a light microscope Zeiss Axio Lab A1 and documented with camera Axio Cam ERc 5.

RESULTS

The testes of the control animals were enveloped by typical dense connective tissue *tunica albuginea* and contained regular seminiferous tubules separated by characteristic interstitium. The seminiferous epithelium was well developed. The lumina of the tubules were filled with continually maturing sperm cells (Fig. 1).

The testes of the experimental animals were covered by *tunica albuginea* with clearly dilated and congested blood vessels. Seminiferous tubules located just beneath the *tunica albuginea* were irregular in shape, and the surrounding interstitium was enlarged and housed highly dilated and congested blood vessels (Fig. 2).

Deeply located seminiferous tubules were similar to these mentioned above and the interstitial blood vessels were highly congested and dilated as well (Fig. 3).

Detailed study of the seminiferous epithelium either from the surface or from deeper areas of the testicular parenchyma showed empty spaces between the developing spermatogenic cells as a result of cellular sloughing. The sloughed immature cells were accumulated within the lumina of the seminiferous tubules. Some locations of the seminiferous tubules possessed an undulated basement membrane (Fig. 4).

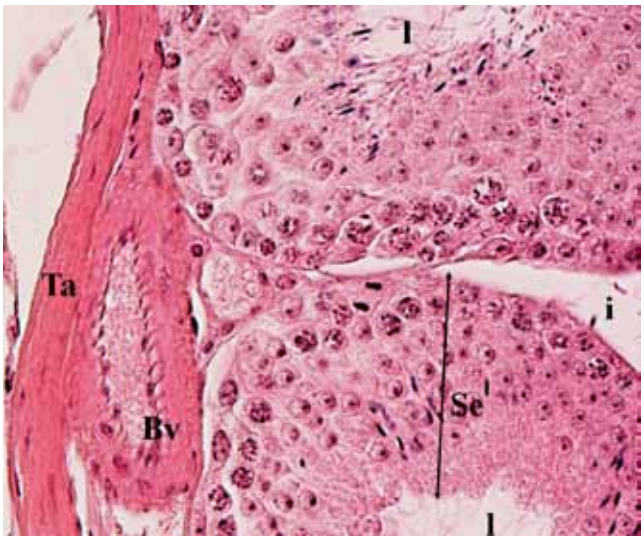

Fig. 1. Surface area of the testis with tunica albuginea in control animal (H & E). Magn. ×400
Ta — *tunica albuginea*; Bv — blood vessel (artery); Se — seminiferous epithelium; l — lumen of seminiferous tubule; i — interstitium

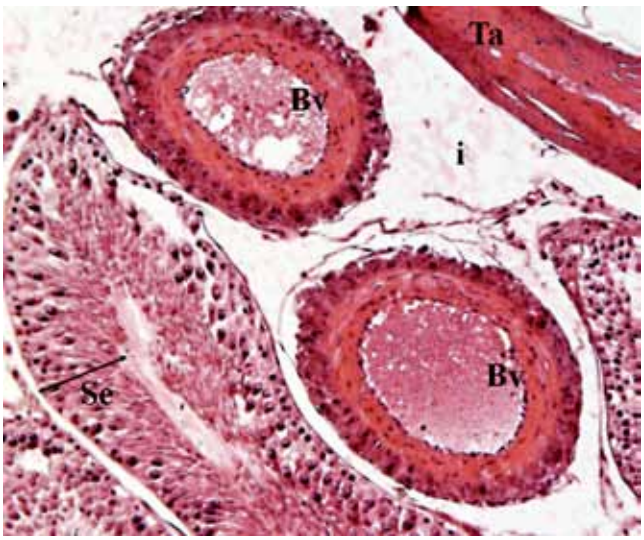

Fig. 2. Surface area of the testis with tunica albuginea in experimental animal after 3 weeks exposure to EMR (H& E). Magn. ×100
Ta — *tunica albuginea*; Bv — dilated and congested blood vessel; Se — seminiferous epithelium; i — interstitium

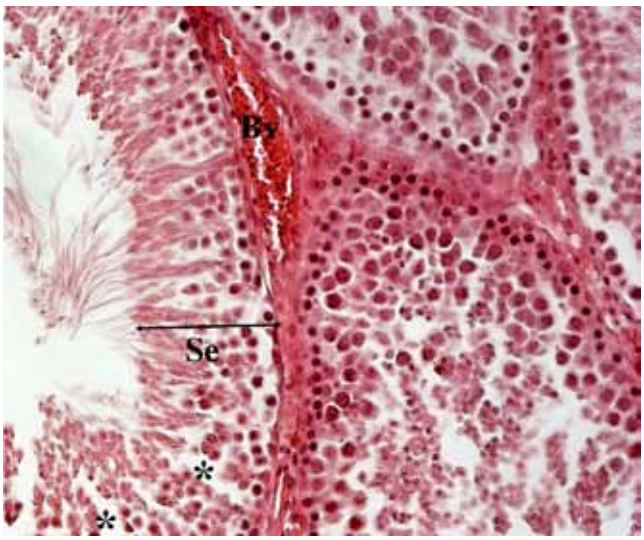

Fig. 3. Deep area of the testis in experimental animal after 3 weeks exposure to EMR (H& E). Magn. × 100
Se — seminiferous epithelium; Bv — dilated and congested blood vessel; * — empty spaces

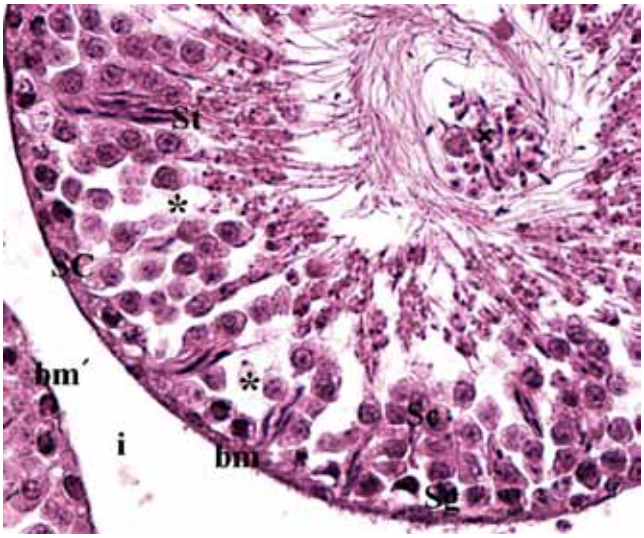

Fig. 4. Detail of the seminiferous tubule from deep area of the testis in experimental animal after 3 weeks exposure to EMR (H & E). Magn. ×400
bm — basement membrane; bm´— undulated basement membrane, SC — Sertoli cell, Sg — spermatogonium; Sc — spermatocyte; St — spermatide; * — empty spaces; ˣ — immature sloughted cells, i — interstitium

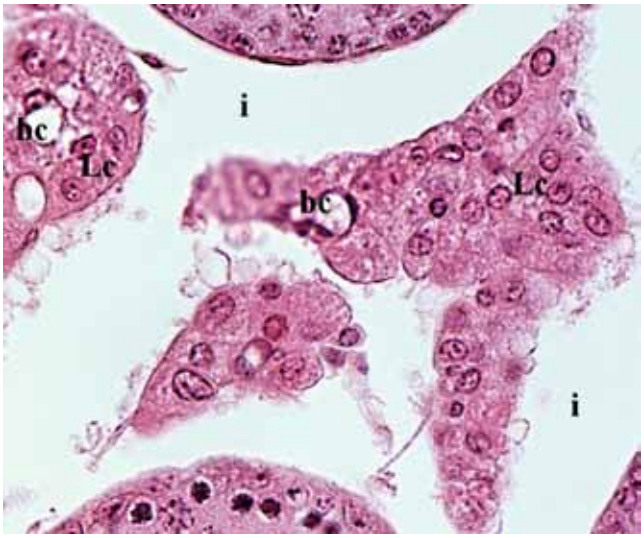

Fig. 5. Interstitial space between seminiferous tubules with clusters of Leydig cells in experimental animal after 3 weeks exposure to EMR (H & E). Magn. ×400
i — interstitial connective tissue, Lc — Leydig cells; bc — blood capillary

The interstitial spaces between the seminiferous tubules were comparably wider than in the control animals. They housed typical large clumps of Leydig cells with the characteristic structure. The associated blood capillaries were typical (Fig. 5).

The *ductuli efferentes* were lined with a ciliated pseudostratified columnar epithelium, and the lumina often contained clumps of immature sex cells sloughed out from the seminiferous epithelium. *Ductuli* were encircled with a regular smooth muscle, and the surrounding interstitium showed no apparent morphological changes (Fig. 6).

The ductus epididymidis had well-preserved histological structures. The pseudostratified columnar epithelium consisted of tall principal cells with numerous stereocilia and shorter basal cells. The lumina contained masses of spermatozoa. The surrounding smooth muscle layer and interstitium showed no abnormal features (Fig. 7).

DISCUSSION

In the past few decades a number of modern electronic devices have been developed by technicians. They are widely used in the broadcasting and communication fields (cellular phones and base stations), in the health care industry, food industry and others. Especially the increased use of wireless

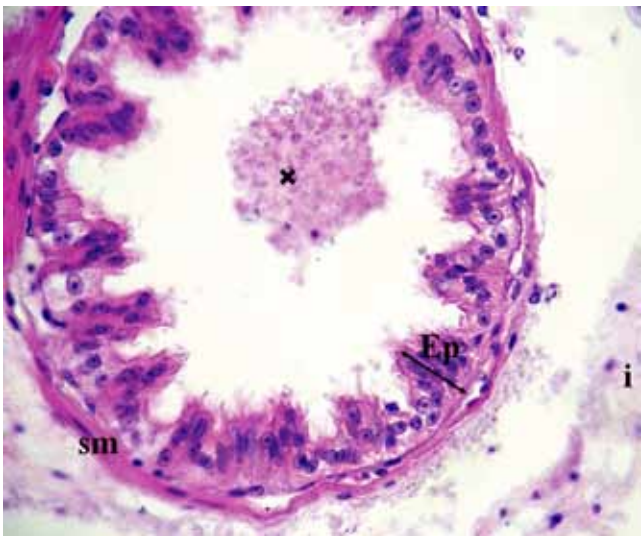

Fig. 6. Efferent duct in experimental animal after 3 weeks exposure to EMR (H & E). Magn. ×250
Ep — pseudostratified columnar ciliated epithelium; sm — smooth muscle layer; × — clumped immature sloughted cells; i — interstitial connective tissue

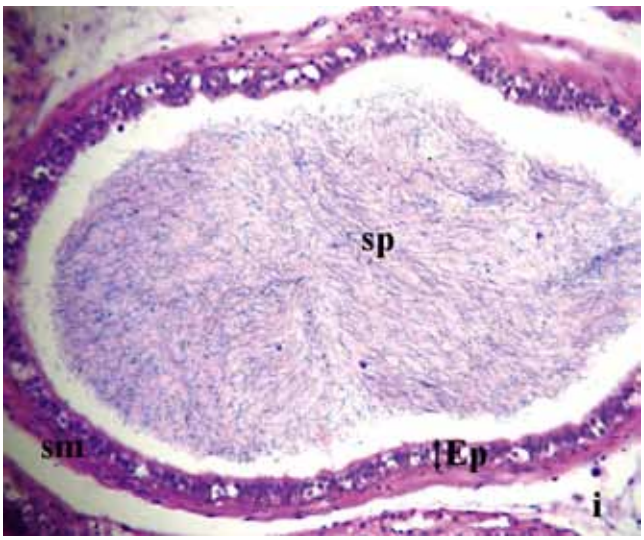

Fig. 7. Ductus epididymidis in experimental animal after 3 weeks exposure to EMR (H & E). Magn. ×150
Ep — pseudostratified columnar epithelium; sm — smooth muscle; sp — masses of spermatozoa; i — interstitial connective tissue

technologies, based on radiofrequency radiation with transmission of microwaves, have resulted in a lot of concern about the possible negative effects on living systems. Lately, it has raised interest about the possible harmful effects of EMR to the reproductive functions, because of the close proximity of the devices to the reproductive organs, and also due to their high sensitivity. In this study, the effect of pulsed EMR on testicular and epididymal morphology was investigated. The results showed that the whole body irradiation at a frequency of 2.45 GHz and a mean power density of 2.8 mW.cm^{-2} for 3 h/day, for 3 weeks, had an adverse effect on the testicular structures in adult rats. Alterations of the shape of the seminiferous tubules accompanied with widening of interstitial space, and different degenerative features in the seminiferous epithelium recorded in our study are consistent with other similar studies [6], [13], [24], [27], [28], [29], [31]. The tissue damage has been attributed to the non-thermal, or a combination of thermal and non-thermal effects of EMR. Several studies have also shown the positive effects of antioxidants such as vitamin C, E, zinc or melatonin, in EMR-induced oxidative stress and apoptosis in the testis [5], [11], [15], [16], [23], [33]. In our work, the interstitial Leydig cells appeared to be unaffected, however, in similar studies, they noted changes in the count of the Leydig cells [7], [14], [31] or testosterone levels [1], [8], [18], [28], probably due to different energy levels of the EMR, or variability time exposure.

Besides the eyes [3], 32], the nervous system [21] and the cardiovascular system [19], [30], [34], the reproductive organs are the most sensitive to EMR (especially at high intensities) where the main effect is thermal injury of the tissue. An increase in the temperature of the gonads causes morphological changes in these organs. This is in agreement with the next obvious positive finding in the present work — the congested and dilated blood vessels within the *tunica albuginea* and testicular interstitium as the sign of the compensation to remove heat buildup. The scientific knowledge proving this phenomenon is very restricted and authors focused mainly on such organs like the heart, kidney or the liver [10], [12], [19], [29]. From this perspective, the present study may be regarded as unique.

The epididymis structure showed no marked histological changes in the present study, except for the occurrence of immature sloughed epithelial cells within the lumina of *ductuli efferentes*. It is known that the spermatozoa are electrically active cells, and from this point of view, we can suspect their high responsiveness to the EMR. Changes in the motility, morphology and count of spermatozoa have been clearly proven by many authors [17], [22], [25].

ACKNOWLEDGEMENT

The study was supported by project VEGA No. 1/0037/12.

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Received February 17, 2014

FOLIA VETERINARIA, 58, 1: 31—36, 2014

PROFILE OF FATTY ACIDS AND LIPID OXIDATION IN PORK AFTER FEEDING LINSEED AND VITAMIN E

Bača, M.[1], Marcinčák, S.[1], Čertík, M.[2], Adamechová, Z.[2]
Nemcová, R.[3], Gancarčíková, S.[3], Popelka, P.[1], Marcinčáková, D.[4]

[1]Department of Food Hygiene and Technology
[2]Department of Biochemical Technology, Faculty of Chemical and Food Technology
Slovak University of Technology, Radlinského 9, 812 37 Bratislava
[3]Department of Microbiology and Immunology
[4]Department of Pharmacology, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81, Košice
The Slovak Republic

slavomir.marcincak@uvlf.sk

ABSTRACT

This experimental study investigated the effects of feeding linseed (*Linum usitatissimum* L.,) and vitamin E on the fatty acid composition, fat susceptibility to oxidation and sensory properties, of chilled and frozen pork. Sixty castrated pigs (Slovak large white × Landrace) (55 ± 3 kg initial body weight) were fed either a control or an experimental diet containing 6 % of extruded linseed (56.8 % of α-linolenic acid according to analysis) and 150 mg.kg^{-1} of vitamin E. The animals were slaughtered at 110 ± 5 kg live weight, after 60 days of fattening. There were no adverse effects of either diet on the pig performance. Feeding linseed to pigs increased the content of n-3 polyunsaturated fatty acids (PUFA) in the loin (*m. longissimus dorsi*) and thigh (*m. semimembranosus*) meat (P < 0.05). The supplementation of linseed led to: a 5-fold higher level of α-linolenic acid, 1.6-fold higher level of eicosapentaenoic acid, and 9-fold higher level of docosapentaenoic acid, compared to the control. Also, the experimental diet produced a decrease in the n – 6/n – 3 ratio compared to the control (P < 0.05). Oleic acid and monounsaturated fatty acids decreased (P < 0.05) after feeding the experimental diet. The oxidative stability of the muscle lipids during storage was not influenced by the linseed and vitamin E diet compared to the control (P > 0.05). There was no deterioration impact on the sensory characteristics of the meat produced.

Keywords: linseed; oxidative stability; pork; PUFA; vitamin E

INTRODUCTION

Fat is an important component of the human diet, but current intake of saturated fatty acids (SFA) compared to polyunsaturated fatty acids (PUFA) are considered too high. Nowadays, the intake of n-3 fatty acids is low because of the decrease in fish consumption and the industrial production of animal feeds rich in grains containing n-6 fatty acids, leading to the production of meat rich in n-6 and poor in n-3 PUFA [7]. The Consumption of n-3 PUFA may provide important health benefits to humans [21]. The deficiency of essential PUFA is manifested by: reduced function of immune system; growth retardation; disturbed reproductive capacity; increased inflammatory conditions; and different disease incidences [2].

In general, meat is the major source of fat in the human diet. Manipulation of the fatty acid composition of muscle and fatty tissues has been of great interest to improve a meat's nutritional value and technological qualities by dietary means [27]. In this context, dietary strategies used to customize fatty acid composition of pig fat and meat have been proven to be very effective, because dietary fatty acids can be incorporated into pig fat with little modification [18]. The n-3 PUFA level can be increased in pork by feeding seed meals or oils with a high content of α-linolenic acid (ALA; 18:3; n-3). Linseed is the richest oilseed source of ALA. Therefore, feeding linseed to pigs has been used to increase the levels of n-3 fatty acids in pork [15, 23]. During fattening with feed supplemented with ALA, the pig's body is able to store about 40 % of the ALA. Of this, 63 % is saved as ALA, 28.5 % is metabolised to eicosap-

entaenoic acid (EPA) and 0.9 % to docosahexaenoic acid (DHA), which indicates that conversion is more efficient in pigs than in humans [12].

On the other hand, an increased content of PUFA has detrimental effects on pork quality and shelf life, because unsaturated fats are more vulnerable to lipid oxidation [25]. Lipid oxidation leads to; an increase in off-flavours, odours and damage to sensitive vitamins. Antioxidants such as vitamin E may delay the onset of pork lipid oxidation when used as an ingredient in the diet. Vitamin E is involved in preventing the development of free radicals in cell membranes *in vivo* and *post mortem* [17]. Vitamin E is usually added in the diet as α-tocopheryl acetate because it cannot be synthesised by animals. The administration of natural or synthetic vitamin E had little impact on growth and did not affect the loin's; quality, pH, colour, or water holding ability of pig meat [3].

In our experiment, the effect of a linseed diet (*Linum usitatissimum* L., variety Flanders) high in α-linolenic acid and synthetic vitamin E as an antioxidant, on the fatty acid composition, oxidative stability and sensory properties of the pork produced was evaluated.

MATERIALS AND METHODS

Animals and diets

The experiment was conducted on the university experimental farm according to the University of Veterinary Medicine and Pharmacy in Košice Animal Ethics Committee and in accordance with applicable legislation [6]. Sixty castrated pigs (Slovak large white × Landrace) with an initial body weight of 55 ± 3 kg were divided into one of the two dietary treatments (30 pigs per treatment). Five animals were kept per pen. The diets fed were a standard growing-finishing diet for pigs. The diets consisted of barley 49.40 %, wheat 24.7 %, soybean meal 12.95 %, maize 9.95 % and vitamin-mineral premix 3 % (Schaumalac M70). The experimental diet (L + E) was supplemented with 6 % of extruded linseed (*Linum usitatissimum* L., variety Flanders, with 56.8 % of α-linolenic acid) and vitamin E (synthetic alpha-tocopheryl acetate) at a level of 150 mg.kg^{-1}. The pigs were fed for 60 days to an average live weight of 110 ± 5 kg. The pigs were provided with feed ad libitum with water available at all times. The individual live weight was recorded at the beginning and at the end of the experiment. Feed intake was measured weekly.

The slaughter processing at a commercial abattoir was conducted on two groups of 30 pigs. After slaughter and overnight chilling, the samples of thigh muscles (*m. semimembranosus*) and loin (*m. longissimus dorsi*) were taken from each carcass. The subcutaneous fat was removed from the muscles. The samples were vacuum packed in polyethylene bags and stored in a refrigerator at 4 °C for 9 days and in a freezer (–18 °C) for 9 months. The stored samples of thighs and loins were analyzed for the determination of: their chemical compositions of fats; extent of fats degradation changes; and sensory evaluation. The samples for the determination of fatty acid profiles were frozen (–18 °C) and stored for one month before performing the analysis.

Analytical determinations

The dry matter was determined by oven drying at 105 °C [1]. The crude protein content was determined using a Kjeltec Auto 1030 (Tecator Co., Sweden). Lipids were isolated in the ground meat samples (*m. semimembranosus*, *m. longissimus dorsi*) with petroleum ether using the Soxhlet apparatus and were then determined gravimetrically.

The fatty acids of the total lipids were analysed as their methyl esters using gas chromatography (GC-6890 N, Agilent Technologies, USA) with a programmed 60 m DB-23 capillary column (Agilent Technologies, USA) according to Čertík et al. [5]. The fatty acid methylester peaks were identified by authentic standards of a C4 to C24 fatty acid methylesters mixture (Supelco, USA).

To determine the lipid oxidation changes of the loin and thigh meat, the method of thiobarbituric acid value determination was used. The examination of the samples was carried out on days 1, 6 and 9 of the storage at chilling conditions (4 °C) and after 1, 6 and 9 months of freezing (–18 °C). The extent of lipid oxidation was evaluated as thiobarbituric acid reactive substances (TBARs) by the method of Marcinčák et al. [13]. The results were quantified as malondialdehyde (MDA) equivalents (mg MDA.kg^{-1} muscle).

Sensory evaluation

A scoring test, paired comparison test [10], was used for the determination of the sensory quality of the meat. Meat samples (thigh and loin) were evaluated 24 hours after slaughtering (4 °C), after 9 days of storage in a refrigerator (4 °C) and within 9 months of storage in a freezer (–18 °C). The samples were thermally treated using the boiling method; kept in hot water until they achieved an internal temperature of 70 °C for 10 minutes. After cooking, the samples were cooled to 40 °C and served to the seven panellists to evaluate the sensory characteristics. The panellists were selected from among the trained laboratory staff. A five point intensity scale for each of the following properties (maximum score 20 points), i. e., taste, aroma, juiciness, and appearance, were applied.

Statistical analysis

Statistical analysis was performed by the statistical software GraphPad Prism (ver. 5, 2007, USA). All data were presented as mean values with the standard deviation (mean ± SD). The results of the chemical composition and fatty acids profiles were compared by the *t*-test. The amount of MDA in the groups during storage and sensory evaluation was compared by a one-way ANOVA analysis of variance. Tukey's multiple comparison test was used to compare statistical differences among the values and the $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Muscle composition

The experimental diet demonstrated no significant changes in the chemical composition regarding the crude protein, total lipids and water compared to the control diet (Table 1). The experimental diet also, had no adverse effects ($P > 0.05$) on the animal growth, feed intake, feed conversion, final weight, and thighs and loin weights (data not shown). The study of Guillevic et al. [7] have shown that feeding linseed to pigs had no detrimental effects on the growth performance.

Table 1. Chemical composition [%] of loin (*m. longissimus dorsi*) and thigh (*m. semimembranosus*) meat

		Water	Fat	Proteins
Loin	Control	73.0 ± 0.60	1.73 ± 0.55	23.43 ± 0.72
	L+E	71.79 ± 1.19	2.15 ± 1.04	22.81 ± 0.83
Thigh	Control	71.28 ± 2.25	3.94 ± 0.65	21.30 ± 1.25
	L+E	70.15 ± 1.44	5.32 ± 0.96	20.46 ± 0.88

L + E — linseed diet + vitamin E

Table 2. Fatty acids composition (%) of muscle fat of loin (*m. longissimus dorsi*) and thigh (*m. semimembranosus*)

Fatty acids [%]	Loin		Thigh	
	L + E	Control	L + E	Control
C 14:0	1.20 ± 0.08	1.38 ± 0.15	1.21 ± 0.05	1.51 ± 0.13
C 16:0	22.66 ± 0.78	23.29 ± 0.86	22.41 ± 0.48	23.65 ± 0.74
C 18:0	12.32 ± 0.50	12.80 ± 0.80	11.40 ± 0.6	12.21 ± 0.70
C 18:1 n-9	42.26 ± 0.49[a]	45.53 ± 0.19	43.3 ± 0.62[a]	46.81 ± 0.92
C 18:1 n-7	3.99 ± 0.41	4.05 ± 0.28	4.47 ± 0.39	3.99 ± 0.50
C 18:2 n-6	6.55 ± 0.79	7.04 ± 0.54	6.71 ± 0.62	6.85 ± 0.75
C 18:3 n-6	0.11 ± 0.01	0.17 ± 0.02	0.10 ± 0.01	0.10 ± 0.01
C 18:3 n-3	3.51 ± 0.20[a]	0.42 ± 0.09	3.15 ± 0.11[a]	0.47 ± 0.12
C 20:0	0.28 ± 0.05	0.21 ± 0.01	0.23 ± 0.02	0.22 ± 0.01
C 20:1 n-9	0.86 ± 0.18[a]	1.06 ± 0.09	0.73 ± 0.2[a]	1.1 ± 0.12
C 20:2 n-6	0.35 ± 0.06	0.41 ± 0.07	0.32 ± 0.12	0.35 ± 0.06
C 20:3 n-6	0.10 ± 0.04	0.11 ± 0.09	0.12 ± 0.02	0.09 ± 0.02
C 20:4 n-6	0.47 ± 0.19[a]	0.65 ± 0.18	0.32 ± 0.17	0.35 ± 0.17
C 20:5 n-3	0.13 ± 0.05[a]	0.09 ± 0.02	0.16 ± 0.02[a]	0.09 ± 0.01
C 22:1 n-9	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
C 22:5 n-3	0.23 ± 0.10[a]	0.03 ± 0.01	0.30 ± 0.03[a]	0.06 ± 0.01
C 22:6 n-3	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.01	0.03 ± 0.01
ΣSFA	38.37 ± 0.76	38.08 ± 0.56	38.28 ± 0.70	38.71 ± 0.34
Σ MUFA	49.64 ± 0.58[a]	52.37 ± 0.61	50.49 ± 0.40[a]	52.89 ± 0.28
ΣPUFA n-3	3.91 ± 0.21[a]	0.78 ± 0.23	3.66 ± 0.11[a]	0.65 ± 0.17
ΣPUFA n-6	8.08 ± 0.32	8.77 ± 0.28	7.57 ± 0.56	7.75 ± 0.82
ΣPUFA	11.99 ± 0.29	9.55 ± 0.26	11.23 ± 48[a]	8.40 ± 0.21
n-6/n-3 ratio	2.06 ± 0.24[a]	11.24 ± 0.15	1.98 ± 0.13[a]	11.92 ± 0.29

L + E — linseed diet + vitamin E; SFA — saturated fatty acids; MUFA — monounsaturated fatty acids
PUFA — polyunsaturated fatty acids; [a] — statistically significant difference compared to control ($P < 0.05$)

Table 3. Determination of TBARs [MDA mg.kg^{-1}] in loin (*m. longissimus dorsi*) and thigh (*m. semimembranosus*) meat stored at chilling conditions [4 °C for 9 days]

Days of storage	L+E		Control	
	Loin	Thigh	Loin	Thigh
1	0.106 ± 0.033	0.116 ± 0.024	0.075 ± 0.020	0.105 ± 0.011
6	0.164 ± 0.041	0.187 ± 0.041	0.155 ± 0.023	0.119 ± 0.010
9	0.312 ± 0.020	0.304 ± 0.055	0.283 ± 0.016	0.232 ± 0.042

L + E — linseed diet + vitamin E

Table 4. Determination of TBARs [MDA mg.kg^{-1}] in loin (*m. longissimus dorsi*) and thigh (*m. semimembranosus*) meat stored at freezing conditions [–18 °C for 9 months]

Months of storage	L + E		Control	
	Loin	Thigh	Loin	Thigh
0	0.108 ± 0.021	0.117 ± 0.033	0.075 ± 0.020	0.105 ± 0.011
6	0.164 ± 0.030	0.218 ± 0.040	0.168 ± 0.027	0.197 ± 0.015
9	0.196 ± 0.014	0.292 ± 0.056	0.188 ± 0.042	0.272 ± 0.043

L + E — linseed diet + vitamin E

Table 5. Overall sensory evaluation of loin (*m. longissimus dorsi*) and thigh (*m. semimembranosus*) meat (maximum score 20 points)

		Chilled, day 1	Chilled, day 9	Frozen, 9 months
Loin	Control	13.85 ± 1.62	13.29 ± 2.84	13.0 ± 1.65
	L+E	14.23 ± 2.59	14.43 ± 2.59	14.1 ± 2.10
Thigh	Control	14.53 ± 2.21	13.92 ± 3.01	12.5 ± 1.90
	L+E	16.46 ± 1.92	15.75 ± 1.81	14.5 ± 1.90

L + E — linseed diet + vitamin E

Fatty acid composition

The fatty acid composition of muscle fat (*m. longissimus dorsi* and *m. semimembranosus*) produced on the L+E diet and the control diet is presented in Table 2. No significant effect of the linseed diet on quantities of SFA was observed, but the monounsaturated fatty acids (MUFA) were reduced in the muscle fats ($P < 0.05$) after linseed feeding. The major MUFA, oleic acid (18:1), was significantly lower in both types of muscles ($P < 0.05$). Juárez et al. [11]also reported a significantly lower proportion of MUFA in the intramuscular fat of loin from pigs fed by linseed (5% or 10%, respectively), compared to the control. In general, meat from pigs fed with linseed resulted in an increase ($P < 0.05$) in total PUFA and n-3 PUFA [24]. In comparison with the control diet, the L+E diet resulted in significantly higher amounts of all n-3 PUFA. The increase in n-3 PUFA levels were on average, 5-fold in the loin and thigh compared to the control; most likely caused by higher levels of ALA. Wiecek and Skomial [26] confirmed the beneficial effect of linseed oil on the fatty acid profile, especially for n-3 PUFA.

There was recorded, a decrease in the level of linoleic acid and a significant decrease in arachidonic acid (AA; 20:4; n-6) ($P < 0.05$),when linseed and vitamin E were fed. Consequently, this led to a linear decrease in the total content of

n-6 PUFA. The decrease in AA levels might be considered beneficial, since increasing concentrations of this fatty acid in membrane phospholipids result in an overproduction of eicosanoids and induce platelet aggregation [20].

An important finding was the 1.6-fold increase in the proportion of EPA and nearly 9-fold increase in docosapentaenoic acid (DPA; 22:5; n-3) in both types of muscles ($P < 0.05$). This was a consequence of the elevated levels of ALA, which served as the precursor for the synthesis of both EPA and DPA. DHA values were not changed when comparing both groups. No effect of linseed on the proportion of DHA was observed also, by Nuernberg et al. [16].

The recommended ratio of n-6/n-3 PUFA in the human diet should be 4/1 [4]. As a result of feeding 6 % linseed, the ratio of n-6/n-3 in the loin and the thigh was approximately 6-fold lower compared to the control. Turner et al. [23] investigated the effect of feeding 5 % and 10 % linseed on the quality of the pork produced and subcutaneous fat. They concluded that linseed dietary supplementation was effective in improving the n-3 PUFA proportion and decreasing the n-6/n-3 ratio. These results mean an increase in the nutritional quality of pork meat for consumers.

Oxidative stability

The results of the oxidation stability in the muscle samples stored at 4 °C are shown in Table 3. Storage in a refrigerator for 9 days resulted in an increase in the amount of MDA in both groups. The values of MDA in the thigh and loin samples of the L + E diet were not significantly different ($P > 0.05$) compared to the control, despite a higher concentration of PUFA. Another study which focused on feeding higher proportion of PUFA without vitamin E, revealed low oxidative stability of meat and meat products [9]. In our study, the addition of vitamin E kept the oxidation of the fat in the muscle at acceptable levels. Similarly, Teye et al. [22] did not find any effect of a higher concentration of PUFA on oxidative stability of meat using a high concentration of vitamin E (250 $mg.kg^{-1}$) in the feed of pigs. Conversely, Guillevic et al. [7] presented significantly higher values of TBARs and reduced oxidative stability in the loin after feeding linseed, but at lower doses of vitamin E (40 $mg.kg^{-1}$).

A similar trend of TBAR values in chilled meat was found, also during freezing storage for 9 months (Table 4). Storage of samples in the freezer did not stop the oxidation process but the amount of degradation products of oxidation of fats in both groups were not higher compared to the chilled meat. TBAR values for all samples (chilled and frozen) did not exceed the values of MDA 0.5 $mg.kg^{-1}$ of meat. While the limit values of MDA in fat are not established by legislation, several authors pointed out that the values above 0.5 mg MDA.kg^{-1} mean advanced process of lipid oxidation and adversely affect the sensory properties of meat [8]. Based on this information, meat produce chilled, as well as, frozen was considered as oxidative stable, without adverse influence of oxidation on the quality of meat.

Sensory analysis

The results of the sensory evaluation of fresh chilled and frozen meat are given in Table 5. Samples of fresh meat from the L + E diet group reached statistically higher scores ($P < 0.05$) compared to the control. There was no effect of the experimental diet, either on the aroma or the overall acceptability of the meat. Our results are comparable to those of Matthews et al. [14], which also determined, that in pigs, no significant differences in meat quality evaluated by trained professionals after feeding 0, 5 or 10 % linseed were found. Another study did not describe any negative impact on the quality of pork after feeding linseed [19]. When evaluating frozen meat, a statistical difference between the diets was found, when the meat of the L + E diet was rated slightly better than the control. Long-term storage seems to have no effect on the deterioration of organoleptic characteristics of meat enriched with linseed.

CONCLUSIONS

The present study demonstrated that supplementation of linseed (6 %) and vitamin E (150 $mg.kg^{-1}$) led to an increase in the n-3 PUFA proportion in loin and thigh meat compared to the control. The n-6/n-3 ratio was greatly reduced with a linseed and vitamin E diet. The adding of vitamin E avoided excessive oxidation in meat enriched with PUFA. Also, there were no deterioration effects on the sensory characteristics of the fresh chilled and frozen meat. It is thus possible to provide for consumers, food with a significantly beneficial impact on human health.

ACKNOWLEDGEMENT

The study was supported by grants VEGA 1/0975/12, 1/0457/14 and 1/0435/11 from the Grant Agency of the Ministry of Education, the Slovak Republic, grant APVV-0662-11 from the Slovak Research and Development Agency, the Slovak Republic, and by the project SK0021, co-financed through the EEA financial mechanism, the Norwegian financial mechanism and the state budget of the Slovak Republic.

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Received March 5, 2014

FOLIA VETERINARIA, 58, 1: 37—42, 2014

IS THERE A RISK OF HEREDITARY EYE DISEASES (HED) IN SLOVAK NATIONAL DOG BREEDS? (A REVIEW)

Mihalová, M., Trbolová, A.

Small Animals Clinic, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
The Slovak Republic

mihalova.martina.mvd@gmail.com

ABSTRACT

During the past few decades, the diseases with genetic backgrounds have become a frequently discussed topic among both the scientific and lay communities interested in dog breeding. Hereditary eye diseases (HED) are an autonomous group of disorders with miscellaneous clinical signs resulting in various severities of visual impairment. These HEDs may be affected by, breed predisposition, age of onset, as well as the type of heredity. Some inherited eye diseases are breed-specific, while in others, typical ophthalmic symptoms could be present in more dog breeds, even if their origin is genetically different. However, there is one common feature, i. e., the incidence of the HED increases as the genetic pool of the breed becomes narrower. Kennel clubs in European and Northern American countries strive to control and eliminate inherited diseases in purebred dogs, including those affecting the eyes. The effort to establish breeding programmes for dog breeds predisposed to HED has become part of the agenda of dog breeding clubs also in Slovakia. Unfortunately, there are no summary reports about the incidences of inherited diseases in the dog breeds of Slovak origin, such as the Slovakian Chuvachs, Czechoslovakian Wolfdogs, Slovakian Hounds or Wire-haired Slovakian Pointers. With reference to the fact that their populations do not have wide breeding pools, despite some dams and sires being bred also in other countries, and with regard to the findings of hereditary eye diseases in the relative dog breeds, there is a suspicion that the Slovak national dog breeds could be at risk of hereditary eye diseases also.

Key words: eye diseases; hereditary; Slovak dog breeds

INTRODUCTION

At present, the FCI (Fédération Cynologique International) recognises 343 dog breeds. Each of them is a "property" of some country where it originated. The "owner" countries of the breeds set standard features for each breed in collaboration with the Standards and Scientific Commissions of the FCI, and the translation and updating are carried out by the FCI continuously [16].

Every dog breed is characterised by its typical appearance and temperament. To sustain those characteristics, there has been a tendency to prefer breeding those individuals which display the most typical characteristics of that breed. Generally, such selection reduces the breeding pool. In the past, inbreeding was common as well. Consequently, dog breeds have become genetically limited populations. Such a narrow group of bred dogs and the frequent use of popular sires, has led to an increased incidence of inherited diseases in populations of some dog breeds [55].

About 430 hereditary diseases have been described in dogs [20]. For only about 60 % of them, is the type of heredity known and of these, 75 % are spread in a recessive manner [55]. Some diseases have been found only in some dog breeds or family lines, so these diseases are presumed to be hereditary [14]. Ackerman [1] reported that inherited diseases are spread rather in family lines, than in the breed itself.

Hereditary eye diseases (known as HED [15] within the international community of veterinary ophthalmologists) consist of a group of disorders that are carefully monitored, not just because they influence the health of individual dogs, but because they spread within the breed itself. The HED group involves disorders present

at birth (e. g. microphthalmia), but also those appearing in adult age (e.g. hereditary cataract, late-onset progressive retinal atrophy). In addition, these HEDs include: sluggish diseases with minimum influence on vision (e. g. mild form of persistent pupillary membrane, multifocal retinal dysplasia); progressive ones seriously impairing vision (e.g. corneal endothelium dystrophy, progressive retinal atrophy); polygenic diseases (e. g. entropium, ectropium); and those transmissible according to simple Mendelian rules (with autosomal recessive/dominant trait) inherited by siblings at high frequency (e. g. prcd form of progressive retinal atrophy) [2], [19], [46], [62], [63], 64].

However, during the past few decades, canine eye diseases presumed to be hereditary became the topic, not only of dog breeders and veterinarians, but even genetic researchers as their incidence has increased worldwide in many dog breeds. On the basis of research, the national kennel clubs attempt to control the prevalence of eye disorders through screening and breeding programmes. The countries leading the investigations toward the elimination of genetically predisposed ocular diseases are Great Britain, USA and Scandinavian countries [5], [7], [12], [25], [36], [55].

The cases from the HED group have been diagnosed also in dogs bred in Slovakia [39], [65]. Moreover, American Cocker Spaniels are required to be examined for hereditary eye diseases if they are involved in breeding [59] in the Slovak Republic and Golden and Labrador Retrievers [60] and all varieties of Collies [27] are recommended to be examined too. The question is: are the HED cases in Slovak purebred dogs, present due to a narrow breeding pool or have they been brought from abroad [38] ? In addition, the second question is: what is the HED situation in Slovak national dog breeds?

There are four dog breeds of Slovak origin recognised by FCI (Slovakian Chuvach, Czechoslovakian Wolfdog, Slovakian Hound and Wire-haired Slovakian Pointer). They have been spread to other countries in rather small numbers, therefore, their populations are relatively small and their genetic pools are relatively narrow. Furthermore, some diseases considered to be influenced by heredity have already appeared in these breeds. For instance, hip dysplasia (HD) has occurred in Slovakian Chuvaches [28], Czechoslovakian Wolfdogs [66] and in some Slovakian Hounds [29]. Accordingly, the suspicion that these breeds could be affected by hereditary eye diseases, is justifiable.

Background and ancestry of Slovak national dog breeds

The formation of dog breeds in Slovakia was influenced by their utilization. In the 16th century, sheepherding grew mainly in the northern regions of the country and shepherd dogs became very important. On the other hand, the woodland character of the country has provided for traditional hunting for centuries. Consequently, hunting dog breeds have been developed.

Slovakian Chuvach

Slovakian Chuvach ("Slovenský čuvač" in Slovak) is classed under the number 142 in the FCI Group 1 — Sheepdogs and Cattle Dogs [17]. At present, Chuvaches are used mainly as yard dogs, rarely as sheepdogs. However, in the past, Slovakian Chuvaches were bred particularly by shepherds in mountainous landscapes to protect herds against predators such as bears and wolves [40].

First evidence about huge white dogs with sharp temperament living in the mountains of the Caucasus, Alps and Pyrenees, date back to 30,000 years ago. They were the ancestors of the local shepherd dog breeds, including Slovakian Chuvach [34].Official references to the Chuvach breed date later, to about the 19th century, when the number of Chuvaches was critically small [40]. In the 1830's, professor Antonín Hrůza, a member of the staff of the Veterinary University in Brno, established an organization for purebreeding of Chuvaches. According to professor Hrůza, the early ancestor of the Chuvach was the large arctic white dog; strong and strict dogs bred by Goths to guard their cattle. They had brought this dog to Pomerania and the Pomeranian White Shepherd and Pomeranian Spitz arose. They spread to the regions of Poland, Slovakia, Carpathian Ruthenia, Hungary, as well as to Italy and France and were crossbred with local dog breeds [24]. Another opinion is, that the Chuvach had originated in the area of central Europe, where it has been developing up to now [40]. It seems that until the 19th century, the Chuvach had been crossbred with; the Pomeranian White Shepherd, Pomeranian Spitz, Komondor, Russian Shepherd Dog, and even the Borzoi. However, the nearest relatives of the Slovak Chuvach are; the Hungarian Kuvasz (Kuvasz), Polish Tatra Shepherd Dog (Polski Owczarek Podhalanski), Italian Maremma and Abruzzes Sheepdog (Cane da pastore Maremmano-Abruzzese), but also white shepherds from Spanish and French Pyrenees — Pyrenean Mountain Dog (Chien de Montagne des Pyrénées) and Pyrenean Mastiff (Mastín del Pirineo) [40].

Czechoslovakian Wolfdog

The second breed of Slovak origin, is the Czechoslovakian Wolfdog ("Československý vlčiak" in Slovak), classified in the FCI Group 1 with registration number 332 [17]. The history of the breed began in the 1950's, in the territory of the former Czechoslovakia, when German Shepherds had been experimentally crossbred with the Carpathian wolf (*Canis lupus lupus*). The experiments ran under the supervision of a lieutenant colonel, (LTc) Karel Hartl, the chief of the Frontier Guards Kennel Club. The primary aim of the crossbreeding was to improve the efficiency of the dogs in service of the Frontier Guards. Later, development of a new dog breed began under the management of LTc Karel Hartl and Major František Rosík [22], [31]. The Czechoslovakian Wolfdog breed was finally acknowledged in 1989 [22], [32]. Generally, Czechoslovakian Wolfdogs are considered to have better stamina and endurance compared to German Shepherds. Also their sight during the night, orientation, hearing and olfaction are considered much better [22].

Another two Slovak dog breeds (Slovakian Hound and Wire-haired Slovakian Pointer) belong to hunting dogs. The Slovakian Hound ("Slovenský kopov" in Slovak) is registered by FCI within Scenthounds (Group 6) under No. 244, while the Wire-haired Slovakian Pointer ("Slovenský hrubosrstý stavač") belongs to Pointing Dogs (FCI Group 7) under FCI registration No. 320 [17], [35].

Slovakian Hound

For centuries, there was a heterogeneous population of hunting dogs in central Europe. The dogs that are used for hunting wild boars in today's Slovak Carpathian Mountains, were derived from Slovakian Hound ancestors. They were selected preferentially with regard to their temperament — to follow game with stamina and hold them back for a long time. Their exterior was not so important. These dogs were black and tan coloured, usually with white

signs. The stabilization of the breed was initiated at the beginning of the 20th century. In 1936, Slovakian Hound breeders meeting and breeding programmes were established under the supervision of two personalities of hunting dogs breeding (Koloman Slimák and Andrej Renča) [34]. A breeding population was selected and so the basis of the Slovakian Hound breed was formed by 26 breeding dogs (14 sires and 12 dams). The genetic variability was narrow, but the population of the breed grew [8]. The Slovakian Hound was acknowledged by FCI in 1963 [34]. According to some sources, the Slovakian Hound could be related to; the Austrian Black and Tan Hound (Brandlbracke), Polish Hound (Ogar Polski), Polish Hunting Dog (Gonczy Polski) or Hungarian Transylvanian Hound (Erdélyi Kopó) [72].

Wire-haired Slovakian Pointer

In the 1940's, Koloman Slimák, a referee of the Slovak Kennel Club, started to select grey wire-haired hunting dogs. Then, the Wire-haired Slovakian Pointer breed was created by crossbreeding of; the Weimaraner, German Wire-haired Pointer and Bohemian Wire-haired Pointing Griffon [30], [66]. The breed development continued up to 1970. In 1983, the Wire-haired Slovakian Pointer was acknowledged. Besides Slovakia, the breed has spread to; the Czech Republic, Switzerland, Germany, Ireland, France and a few dogs are bred also in; Austria, Holland, Finland, Great Britain and the United States. Unfortunately, there is no great interest in breeding Wire-haired Slovakian Pointers in Slovakia at the present and there are only 30 breeding individuals [66]. The narrow genetic pool could be increased again by the crossbreeding of the ancestral breeds [33], [66].

Hereditary eye diseases affecting the breed relatives to the Slovakian Chuvach

There are no records about any eye diseases presumed to be hereditary, diagnosed in the Slovakian Chuvach. However, eye diseases considered to be hereditary were found in their ancestors or sibling breeds.

Generally, Tatra Shepherd Dogs and Kuvasz are considered to be sibling breeds with the Slovakian Chuvach. The Tatra Shepherd showed a predisposition only to the ectropium of the lower eyelids [10]; compared to Kuvasz dogs in which, severe ocular disease, prcd, affecting retina and terminating with blindness, has been revealed. The form of canine progressive retinal atrophy (PRA) was proven to be an autosomal recessive in the breed and a genetic test was developed [56], [73]. Besides the prcd, the American College of Veterinary Ophthalmologists (ACVO) has registered distichiasis with 4 % of prevalence and 4—5 % prevalence of persistent pupillary membrane (PPM) in Kuvasz dogs in the USA [2]. The ACVO detected also, the cases of corneal dystrophy (epithelial, stromal and endothelial) [2], [9]. Furthermore, a few cases of hereditary juvenile cortical cataract have been found in the breed [52].

Other dog breeds with similar physiognomy as the Slovakian Chuvach, belonging to the same FCI group, but without proven relationships are; the Pyrenean Mountain Dog (in the USA called Great Pyrenees) and the Maremma Sheepdog. The head shape of Pyrenean Mountain Dog predisposes the eyelids to conformational deformities. The dogs usually suffer from forming a medial canthal pocket of the lower eyelid, sometimes with nonspastic entropium, entropium of the lateral canthus, or the kink in the central part of the eyelid tarsal plates accompanied by entropium medial and lateral to this [50]. The eye diseases proven to be hereditary in Pyrenean Mountain Dog are; distichiasis, hereditary cataract and persistent pupillary membrane which affect almost 25 % of the breed in the USA. ACVO [2], [4], [41] also have referred to cases of PRA, focal and geographic retinal dysplasia (RD).

There is a suspicion that the ancestors of the Slovakian Chuvach used to be crossbred occasionally with Komondors [24] and with Borzois, and so there could be, in theory, the transfer of genotype. There is evidence that some cases of ectropium or entropium in Komondors are presumed to be hereditary [2]. Also, persistent pupillary membrane (PPM) with 1.5 % prevalence in the USA [2], and hereditary cataract (HD) have been diagnosed in the Komondor breed [2], [51]. Furthermore, Borzoi is the breed with high occurrences of hereditary eye diseases, such as; hereditary cataract, persistent pupillary membrane, micropapilla and optic nerve hypoplasia, which often present together as Multiple Ocular Defects syndrome [2], [47]. In addition, the Borzoi breed is affected by; plasmoma, the chronic inflammation of the anterior surface of the nictitating membrane [2], [47], and by multifocal chorioretinopathy (Borzoi retinopathy), the multiple foci of retinal degeneration [47], [57], [61].

Hereditary eye diseases affecting the ancestors of the Czechoslovakian Wolfdog

There are no references about hereditary eye disease in the Carpathian wolf which is one of the ancestors of the Czechoslovakian Wolfdog. On the other hand, the German Shepherd breed suffers from many health disorders including those affecting their eyes. The most frequent ocular disease in German Shepherds is chronic superficial keratitis, generally called pannus [2], [21]. It is a bilateral inflammatory disease of the cornea, where granular tissue containing lymphocytes and plasma cells [70] overgrows the corneal stroma, starting from the ventrolateral part of the limbus and spreads centrally. It is followed by vascularisation and pigmentation of the affected part of the cornea [6], [13], [48]. Chronic superficial keratitis can be associated with plasma cell infiltration of the nictitans membrane [2]. The disease is an autoimmune phenomena [26], [71]. It is known that ultraviolet radiation worsens the clinical signs, so the affected dogs get worse in the summer and on sunny days in the winter and in high altitudes [6], [11], [58]. If the disease is not treated, there is a severe vision impairment due to the overgrowth of the cornea. It is necessary to treat the affected eyes throughout life and to administer immunosuppresive topical drugs, such as dexamethasone or cyclosporine [69]. If the affection of the cornea is extensive, keratectomy is provided [21].

There are two types of hereditary cataracts reported in the German Shepherd breed. The first was found by von Hippel in Germany in 1930 [67]. The cataract was congenital, with opacities found in the anterior capsule, subcapsular zone, anterior cortex and the nucleus. The condition was non-progressive and the equatorial zone remained unaffected. Test breeding indicated an autosomal dominant trait of inheritance. The second type of inherited cataract has been reported by Barnett [3]. There were small vacuoles in the posterior cortex apparent at the age of 3 months and the mode of inheritance was ascertained to be autosomal recessive.

Other inherited eye diseases found in German Shepherds are; corneal dystrophy, persistent pupillary membrane, multifocal reti-

nal dysplasia, progressive retinal atrophy and optic nerve hypoplasia [2], [3], [48]. There is also a reference that German Shepherds could be prone to lens luxation when the incidence is found in family lines [23], [37].

Furthermore, luxation of the lens has been registered in the Dutch population of the Czechoslovakian Wolfdog breed [73] and bilateral lens luxation not connected to other health disorders has occurred also in Czechoslovakian Wolfdog in Slovakia [38]. Also, other hereditary eye disorders were found in the Czechoslovakian Wolfdogs bred in the Netherland (corneal epithelial dystrophy, persistent pupillary membrane, progressive retinal atrophy, multifocal retinal dysplasia and hereditary cataract) [72].

Hereditary eye diseases affecting the breeds related to the Wire-haired Slovakian Pointer and Slovakian Hound

There is only one report of a HED incidence in Wire-haired Slovakian Pointers bred in the United Kingdom, but none in dogs bred in Slovakia. The report has mentioned just one case of strabismus [45]. On the other hand, the ancestral breeds, such as, Weimaraner, German Wire-haired Pointer, and Bohemian Wire-haired Pointing Griffon, have suffered from some inherited eye diseases. In all three breeds, entropium has been reported [2], [49], [53], [54]. The Weimaraner breed suffered also from; medial canthal pocket syndrome, eversion of the third eyelid cartilage, distichiasis, corneal dystrophy and persistent pupillary membrane [2], [53]. Persistent pupillary membrane has been found in the German Wire-haired Pointer breed [2] and distichiasis and corneal dystrophy in the Wire-haired Pointing Griffon breed [2], [54]. Hereditary cataracts, with opacities in the cortex and nucleus, were reported in the past in Weimaraners [2], [53]. Posterior polar subcapsular [49] and anterior, as well as, posterior cortical cataracts [2] have been found in German Wire-haired Pointers and anterior cortical cataracts in Wire-haired Pointing Griffons [54]. Moreover, multifocal retinal dysplasia was reported in the German Wire-haired Pointer breed [2], [49], geographic retinal dysplasia in Wire-haired Pointing Griffon [2], and progressive retinal atrophy in Weimaraners [43].

There are no references about inherited eye diseases in the Slovakian Hound. Also, the related breeds, such as Austrian Black and Tan Hound [18] or Transylvanian (Hungarian) Hound [42] are considered relatively healthy breeds. Just in Polish Hounds [44], [73] and Polish Hunting Dogs [68], [72] there were sporadic palpebral disorders, primarily entropium.

CONCLUSION

The Slovakian Chuvach, Czechoslovakian Wolfdog, Slovakian Hound and Slovakian Wire-haired Pointer are dog breeds of Slovak origin kept mainly in Slovakia. Czechoslovakian Wolfdogs are also kept in the Czech Republic. They are bred in other countries only in small numbers. Because of such narrow breeding pools and limited knowledge about the prevalence of hereditary eye diseases (HED) in these breeds, there is a recommendation that the examination of HED should be included in the breeding rules.

ACKNOWLEDGEMENT

The article has been created with the support of VEGA 1/1192/12 funding.

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Received, February 17, 2014

FOLIA VETERINARIA, 58, 1: 43—46, 2014

CORROSION TECHNIQUE IN THE STUDY OF ARTERIAL VARIATIONS AS THE RESULT OF A CROSS OF THE SLOVAK GREY-BLUE REX AND BLUE OF VIENNA RABBITS

Maženský, D.[1], Petrovová, E.[1], Supuka, P.[2], Supukova, A.[3]

[1]Department of Anatomy, Histology and Physiology
[2]Institute of Nutrition, Dietetics and Feed Production
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
[3]Institute of Experimental Medicine
Faculty of Medicine, UPJŠ Košice, Trieda SNP 1, 041 01 Košice
The Slovak Republic

mazenskyd@gmail.com

ABSTRACT

The aim of this study was to describe the arterial variations of the pelvic cavity and the proximal part of the pelvic limb in 20 hybrids obtained by the crossing of the Slovak Grey-blue Rex and Blue of Vienna rabbits. We prepared corrosion casts of the arterial system of rabbits using Spofacryl. The presence of bilateral *arteria iliaca communis* and its division into *arteria iliaca externa* and *interna* was found in 75% of the cases. In 25% of the cases, we found only the *arteria iliaca* on each side. The *arteria femoralis* ran distally on the medial surface of the femur in 75% of the cases, but in 25% of the cases it ran on its caudal surface. The *arteria circumflexa ilium profunda* branched from the *arteria iliaca externa* on both sides at the same level in 75% of the cases. *The arteria circumflexa ilium profunda* was the first branch to arise from the *arteri iliaca* in 25% of the cases. It was found in 80% of the cases with an atypical arrangement as the branches arose at different levels on each side. In 20% of the cases with atypical arrangements, the left one branched from the *aorta abdominalis* and the right one from the corresponding *arteria iliaca*. This is the first work dealing with the arterial variations in crossbreds of two specific rabbit breeds: Slovak Grey-blue Rex and Blue of Vienna rabbits.

Key words: anatomic variation; arteries; corrosion casting; crossbreeding; rabbits

INTRODUCTION

The knowledge of anatomical variations is important for radiological and surgical procedures in humans and animals due to its practical and theoretical significance for experimental research and surgical practice in experimental and domestic animals [5], [7], [11].

Rabbits have been used as experimental models in many diseases [1], [10]. They have been used for the study of physiology, pathophysiology, toxicology, pharmacology and surgery for the veterinary medicine courses.

Despite the intensive use of rabbits as experimental animals, some aspects of their macroanatomy in the vascular system needs a more detailed description. The aim of this study was to describe some arterial peculiarities of the pelvic cavity and proximal part of the pelvic limb in crossbreds, obtained by the cross of the Slovak Grey-blue Rex and the Blue of Vienna rabbits.

MATERIALS AND METHODS

This study was carried out on 20 adult rabbits (age 140 days). We used rabbits of both sexes (female n = 10; male n = 10) with an average weight of 2.5—3 kg, which were obtained by the cross of the following two breeds: Slovak grey-blue Rex and Blue of Vienna rabbits, in an accredited experimental laboratory of the University of Veterinary Medicine and Pharmacy in Kosice. The animals were kept in cages under standard conditions (temperature 15—20 °C,

Fig. 1. The typical arrangement of the termination of the *aorta abdominalis*
1 — *aorta abdominalis*; 2 — *arteria iliaca externa sinistra*; 3 — *arteria iliaca externa dextra*; 4 — *arteria circumflexa ilium profunda sinistra*; 5 — *arteria circumflexa ilium profunda dextra*; 6 — *arteria iliaca interna sinistra*; 7 — *arteria iliaca interna dextra*; 8 — *arteria sacralis mediana.*
Macroscopic image, ventral view

Fig. 2. The irregular origin of bilateral *arteria circumflexa ilium profunda*
1 — *aorta abdominalis*; 2 — *arteria iliaca sinistra*; 3 — *arteria iliaca dextra*; 4 — *arteria circumflexa ilium profunda sinistra*; 5 — *arteria circumflexa ilium profunda dextra.*
Macroscopic image, ventral view

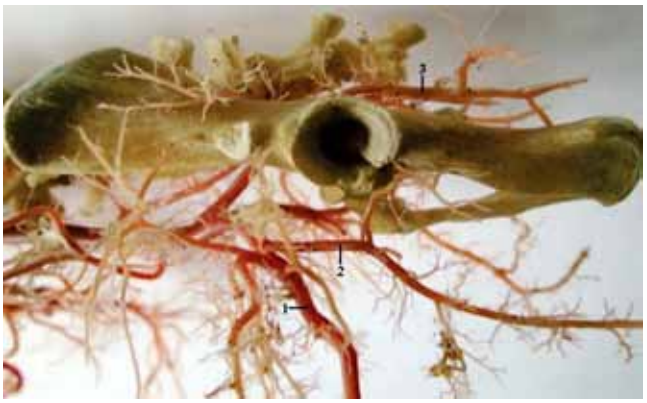

Fig. 3. The typical course of *arteria femoralis*
1 — *arteria femoralis sinistra*; 2 — *arteria profunda femoris sinistra*; 3 — *arteria iliaca interna sinistra.*
Macroscopic image, lateral view

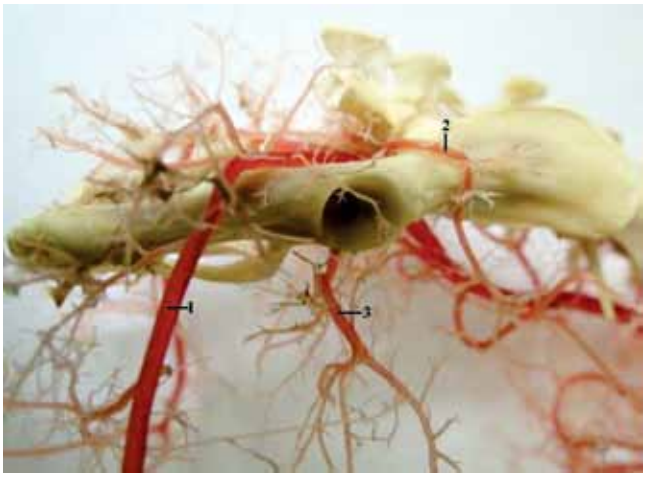

Fig. 4. Independent origin of *arteria circumflexa femoris lateralis dextra* from *arteria iliaca dextra*
1 — *arteria femoralis dextra*; 2 — *arteria glutea cranialis dextra*; 3 — *arteria circumflexa femoris lateralis dextra.*
Macroscopic image, lateral view

relative humidity 45 %, 12-hour light period), and fed with a granular feed mixture (O-10 NORM TYP). Drinking water was available for all animals *ad libitum*. The animals were injected intravenously with heparin (50,000 IU.kg^{-1}) 30 min before they were sacrificed by an intravenous injection of embutramide (T-61, 0.3 ml.kg^{-1}). Immediately after death, the vascular network was perfused with a physiological solution. During the manual injection through the ascending aorta, the right atrium of the heart was opened in order to lower the pressure in the vessels to ensure a well perfused injection [9]. Spofacryl (polymethylmethacrylate, Spofa Dental, Czech Republic) in a volume of 35 ml was used as the casting medium. The maceration was carried out in 2—4 % KOH solution for a period of 5 days at 60—70 °C. This study was carried out under the authority of decision No. 2647/07-221/5.

RESULTS

In the crossbreeds obtained, we found variable arterial arrangements in the pelvic cavity and proximal part of the

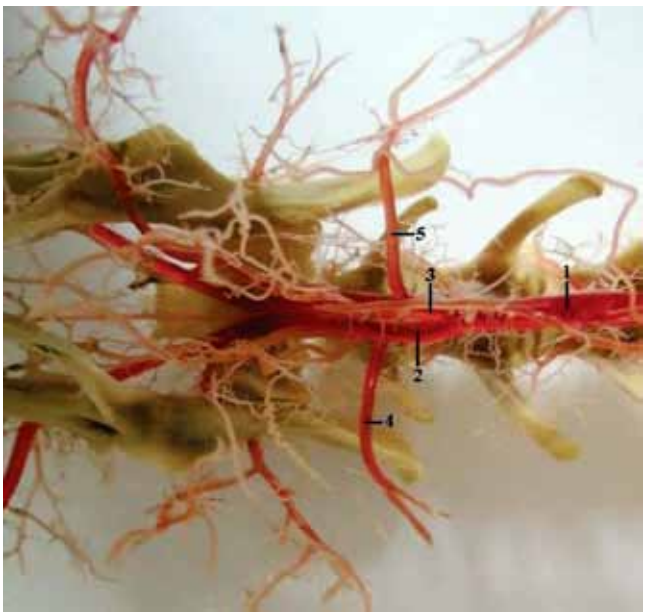

Fig. 5. The origin of bilateral *arteria circumflexa ilium profunda* from *arteria iliaca*
1 — *aorta abdominalis*; 2 — *arteria iliaca sinistra*; 3 — *arteria iliaca dextra*; 4 — *arteria circumflexa ilium profunda sinistra*; 5 — *arteria circumflexa ilium profunda dextra*.
Macroscopic image, ventral view

Fig. 6. The atypical course of *arteria femoralis* and atypical origin of *arteria circumflexa femoris lateralis sinistra*
1 — *arteria femoralis sinistra*; 2 — *arteria glutea cranialis sinistra*; 3 — *arteria circumflexa femoris lateralis sinistra*.
Macroscopic image, lateral view

pelvic limbs. The a*orta abdominalis* was divided typically at the level of the sixth lumbar vertebra into two *arteriae iliacae communes* in 7 % of the cases (Fig. 1) and atypically into two *arteriae iliacae* in 25 % of the cases (Fig. 2). Each of the *arteria iliaca communis* was terminally divided into the *arteria iliaca interna externa* (Fig. 1). The bilateral *arteria iliaca interna* continued on the roof of the pelvic cavity caudally and gave off some branches to the walls and organs of the pelvic cavity (Fig. 3). The a*rteria iliaca externa* was determined for the free pelvic limb and terminally divided into the *arteria femoralis* and *arteria profunda femoris*. The a*rteria femoralis* continued along the medial surface of the femur distally (Fig. 3) and the *arteria profunda femoris* was directed caudally along the femur. The a*rteria iliaca* continued directly caudally on the roof of the pelvic cavity to the level of acetabulum (Fig. 2). It then turned laterally on the caudal aspect of the acetabulum and continued distally along the caudal surface of the femur to the pelvic limb as the *arteria femoralis* (Fig. 4). The a*rteria circumflexa ilium profunda* branched from the *arteria iliaca externa* on both sides at the same levels in 75 % of the cases (Fig. 1). *The arteria circumflexa ilium profunda* was the first branch arising from the *arteria iliaca*. It was found in 80 % of the cases with an atypical arrangement as branches arose at different levels on each side (Fig. 5). In 20 % of the cases, the left one branched from the *aorta abdominalis* and the right one from the corresponding *arteria iliaca* (Fig. 2). We found, also variations in the origin of the *arteria circumflexa femoris lateralis* in the corrosion casts with atypical arrangements. This was present in 60 % as a branch arising from the *arteria iliaca* (Fig. 4). The left one originated in 40 % of the cases from the *arteria glutea cranialis* (Fig. 6) and the right one from the *arteria iliaca* also in 40 % of the cases.

DISCUSSION

In all cases we found the terminal division of the *aorta abdominalis* at the level of the sixth lumbar vertebra. Craigie [4] described this division at the level of the seventh lumbar vertebra. The origin of the bilateral *arteria iliaca communis* at this level was found in 75 % of the cases. The same schema was described also by: Craigie [4], Nejedlý [6], and Popesko et al. [8]. In 25 % of the cases we found the terminal division of the *aorta abdominalis* into bilateral *arteria iliaca artteries*. The *iliaca communis* was divided into the *arteria iliaca externa* and the *arteria iliaca interna* on each side, which agrees with the findings of: Craigie [4], Nejedlý [6], and Popesko et al. [8], but the *arteria iliaca* was missing in this division. The a*rteria iliaca* along its course inside the pelvic cavity gives off the same branch as the *arteria iliaca interna*. The a*rteria iliaca externa* was terminally divided into the caudally directed *arteria profunda femoris* and distally directed *arteria femoralis* which had a medial position to the femur. The same arrangement was described also by: Craigie [4], Nejedlý [6], and Popesko et al. [8]. The a*rteria iliaca,* after leaving the pelvic cavity caudally to the acetabulum, continued distally as the *arteria femoralis*, but along the caudal border of the femur.

The a*rteria circumflexa ilium profunda,* as a branch arising typically from the *arteria iliaca externa* in 75 % of the cases, had its origin at the same level. It had an atypical point of origin in 25 % of the cases. The right one originated from the corresponding *arteria iliaca* and the left one from the *aorta abdominalis*. The a*rteria circumflexa femoris lateralis* typically arose from the *arteria femoralis* [4], [6], [8]. The same situation we found in 75 % of the cases. We found two types of origin of this artery in cases with atypical arrangements. In the first type, it originated from the *arteria iliaca* in 60 % of the cases. In the rest of the cases, the left-sided artery originated from the *arteria glutea cranialis* and the right-sided from the *arteria iliaca*.

CONCLUSIONS

Up until now, published works dealing with the variations in the rabbit arterial system were concentrated only on variations in one specific breed [2], [3], [6]. This is the first work dealing with the arterial variations in crossbreeds obtained by a cross of two specific rabbit breeds: Slovak Greyblue Rex and Blue of Vienna rabbits. Based on our results, it can be suspected that these variations may be linked to the presence of a particular gene. Our study indicates the need for more detailed knowledge of the blood supply in laboratory animals. This would allow more objective results to be gained from fewer experimental animals.

ACKNOWLEDGEMENTS

The present study was carried out within the framework of the project VEGA No. 1/0111/13 of the Slovak Ministry of Education.

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Received April 23, 2014

FOLIA VETERINARIA, 58, 1: 47—52, 2014

EFFICACY OF ANTISEPTICS AGAINST THE YEAST *MALASSEZIA PACHYDERMATIS*

Váczi, P.[1], Čonková, E.[1], Mártonová, M.[2], Sihelská, Z.[3]

[1]Institute of Pharmacology, Department of Pharmacology and Toxicology
[2]Institute of Biochemistry, Department of Chemistry, Biochemistry and Biophysics
[3]Institute of Microbiology and Gnotobiology, Department of Microbiology and Immunology
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

peter.vaczi@uvlf.sk

ABSTRACT

We determined the efficacy of antiseptic agents commonly used in a veterinary practice against *Malassezia pachydermatis* (19 isolates and 1 reference strain). The study was performed to determine the suitability of conventional antiseptics for use as supportive therapy against infections caused by yeasts. *M. pachydermatis* isolates were obtained from the skin and external ear canals of 19 dogs of various breed and age with confirmed mycotic infections (dermatitis and otitis externa). The modified disc diffusion method M44-A2 was used to investigate the efficacy of the chosen antiseptic agents against *M. pachydermatis* strains. We ascertained that there was 100 % effectiveness of 3 % hydrogen peroxide, tincture of iodine, and benzododecine. Lower antifungal activity was recorded for: Lugol's iodine (60—70 %), copper sulphate (bluestone) (60—80 %), and chlorhexidine (60 %). The efficacy of the other tested substances (potassium permanganate, povidone iodine, salicylic acid, ethanol, propanol, thymol and carbethopendecine) was insufficient (≤ 30 %). Our results indicated that use of certain antiseptic agents could help reduce the incidence of fungal infections of the skin and external ear canals in dogs.

Key words: antiseptic agents; disc diffusion method; *Malassezia pachydermatis;* yeasts

INTRODUCTION

An antiseptic is a chemical agent that reduces the microbial population on the skin and other living tissues. Since its mechanism of action involves nonspecific disruption of cellular membranes or enzymes in most cases, caution must be taken not to harm the host tissues. An ideal antiseptic would have: a broad spectrum of activity; low toxicity; and high penetrability. In addition, it would maintain activity in the presence of pus and necrotic tissue, and would cause little skin irritation or interference with the normal healing process [12].

The genus of *Malassezia* species comprise several lipophilic yeasts, normally present on the skin of many warm-blooded vertebrates, including man. The lipid-dependent yeasts, which require lipid supplementation for growth, corresponded to six species, namely; *Malassezia furfur, M. globosa, M. obtusa, M. restricta, M. sloofiae,* and *M. sympodialis*. Lately four new lipid-dependent species have been identified; *M. dermatis, M. japonica, M. nana* and *M. yamatoensis* [4]. Recently, three new species have been characterized; *M. caprae, M. equina* [2], and *M. cuniculi* [3]. The non-lipid-dependent *Malassezia* isolates, which are able to grow on routine laboratory media, were assigned to a single species, *Malassezia pachydermatis*. *Malassezia pachydermatis* is most frequently isolated from; the skin surface, muzzle and jaw zones, external ear canal, anal sacks, vagina, interdigital spaces and rectum, where it is

considered a potential pathogen [7]. It is generally agreed that the dermatohistopathological features of *Malassezia* dermatitis include an irregularly hyperplastic and spongiform superficial perivascular interstitial dermatitis, wherein parakeratotic hyperkeratosis and lymphocytic infiltrations are prominent [15].

Dogs are the most frequently affected animals and the infection may acquire local or generalized form. The local infection affects; the extremities (*pododermatitis*), external ear canal (*otitis externa*), head, neck, axillae and the perianal zone. Its manifestations are; skin erythema, scaliness, and hyperpigmentation. Manifestations of the generalized form include; seborrhoeic dermatitis, folliculitis, furunculosis, exfoliative erythema and lichenized alopetic zones [9]. It occurs most frequently as a secondary infection in atopic patients [21].

Therapy of mycotic infections includes: local application of antifungal preparations containing antiseptics and azole antifungals (ketoconazole); oral administration of antifungals (ketoconazole, itraconazole, fluconazole); and, in case of bacterial infection, suitable therapy involves also systemic antibiotics [13].

Up to now, we have more detailed knowledge only about some preparations with antiseptic effect (chlorhexidine) regarding *M. pachydermatis*. The aim of this study was to investigate the effect of additional antiseptics, in order to extend the range of supportive antifungal therapy.

MATERIALS AND METHODS

The efficacy of selected antiseptics was determined by the modified disc diffusion method M44-A2 [5]. The method is based on pathogen growth inhibition, manifested by the development of a diffusion zone due to the release of the tested antifungal substances from impregnated paper discs. The discs impregnated with 10 µl of respective antiseptic agents were placed onto the surface of inoculated nutrient media. Cultivation took place at a constant temperature of 32 °C for 72 and 96 hours. Then the size of the inhibition zones were measured.

The activities of the following antiseptic agents were investigated: group of oxidative substances — 3 % hydrogen peroxide; 0.2 % potassium permanganate; iodine containing compounds — Lugol's iodine (1 % iodine and 2.5 % potassium iodide); tincture of iodine (6.5 % iodine and 2.5 % potassium iodide); 10 % povidone iodine; organic acids — 5 % and 10 % salicylic acid; 2 % and 5 % propionic acid, 0.5 %, 1 % and 5 % acetic acid; alcohols — 70 % ethanol and 70 % propanol); phenols — 0.5 % thymol; quaternary ammonium compounds — 0.5 % benzododecine and 0.8 % carbethopendecine; biguanides — 2.2 % chlorhexidine; and heavy metal compounds — 5 % copper sulphate (bluestone). Individual antiseptics and their concentrations were selected on the basis of their usability in veterinary practice. All of them could be applied to the skin or to accessible mucous membranes.

M. pachydermatis isolates (n = 19) were obtained from skin swabs and swabs of the external ear canal of dogs of various breeds (Irish Setter, Gordon Setter, English Cocker Spaniel, German Shepherd, Bichon) and age (1—8 years) with confirmed mycotic infection.

Using pure 96-hour cultures passaged on Sabouraud dextrose agar with chloramphenicol — SCH (HiMedia Laboratories Pvt. Ltd., Mumbai, India), a suspension of concentrated 10^6 CFU.ml^{-1} in saline solution, corresponding to standard 1 on McFarland scale, was prepared by densitometer (Pliva — LaChema, Brno, Czech Republic). This suspension was inoculated on to the surface of Petri dishes containing SCH agar, twice in three directions, 15 minutes apart. Twice tested reference strain *Malassezia pachydermatis* IHEM 19215 (Liège, Belgium) was used as a control. Since the method M44-A2 doesn't include interpretation criteria for *Malassezia* strains, those isolates which showed inhibition zones greater or equal to 15 mm (2.5-fold larger than disc diameter) were considered sensitive.

RESULTS

Table 1 shows the results of the antiseptic efficacy from the group of oxidants and halides. Hydrogen peroxide appeared most suitable as it was capable of inactivating 100 % of the tested strains after 72, as well as, after 96 hours. Potassium permanganate in the concentration mentioned above was ineffective against all tested strains. From the iodine compounds group, 100 % activity was reached with tincture of iodine, even after 96 hours and excellent results were obtained also with water solution of iodine — Lugol's iodine. Povidone iodine testing showed its ineffectiveness.

The organic acids tested in the study, salicylic, acetic and propionic, appeared unsuitable and their effect was minimal or none (Table 2).

Regarding alcohols and phenol derivatives, tests were conducted with 70 % ethyl-alcohol, 70 % propyl-alcohol and 0.5 % alcohol solution of thymol but their inhibitory action was negligible. Fungicidal activity (60—80 %) of copper sulphate pentahydrate solution was also confirmed in this study (Table 3).

Results of the efficacy testing of benzododecine, carbethopendecine and chlorhexidine against the *Malassezia* isolates are presented in Table 4. Benzododecine exhibited excellent (100 %) activity while the effect of carbethopendecine was insignificant (30 %). Chlorhexidine in 2 % concentration was effective against 60 % of the strains tested in this study.

DISCUSSION

The use of disinfectants and antiseptic preparations in veterinary medicine is one of the most important preventive measures as they inactivate and destroy protozoa, bacteria, viruses, micromycetes and yeasts found on objects, skin and mucous membranes. The term disinfection means revitalization of micro-organisms in the external environment (objects, floor, walls) while antisepsis involves reduction of micro-organisms on the skin and mucosal surfaces. Antiseptics may exhibit excellent microbistatic and even microbicidal action, but they always have, more or less, toxic and irritating effects on the skin and mucosa of the patient. For this reason, general conditions were set for the use of antiseptics including the type of the compound, its concentration in the solution and eventual possibilities of combining several substances.

Table 1. Efficacy of oxidants and halides against the tested yeasts

Compound/ concentration	Hydrogen peroxide 3 %		Potassium permanganate 0.2 %		Lugol's solution		Tincture of iodine		Povidone iodine	
Exposure	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h
Isolates of *Malassezia pachydermatis* (n = 19)										
x	35.8	32.6	0	0	15.7	14.5	25.6	23.1	7.8	6.9
Min-max	24—55	22—55	0	0	10—20	10—17	22—30	18—27	0—13	0—12
SD	11.85	11.53	0	0	3.13	2.22	2.55	2.69	5.49	4.91
Efficacy [%]	100	100	0	0	70	60	100	100	0	0
***Malassezia pachydermatis* IHEM 19215**										
x	24	20.5	0	0	17	16	29	23	4	0
Min-max	22—26	20—21	0	0	14—20	15—17	28—30	22—24	0—8	0
SD	2	0.5	0	0	3	1	1	1	4	0
Efficacy [%]	100	100	0	0	50	100	100	100	0	0

Abbreviations:
x — mean size of inhibition zones in millimetres; min-max — minimum and maximum size of inhibition zones in millimetres
SD — standard deviation; n — number of tested strains

Table 2. Efficacy of organic acids against the tested yeasts

Compound/ concentration	Salicylic acid				Propionic acid				Acetic acid					
	5 %		10 %		2 %		5 %		0.5 %		1 %		5 %	
Exposure	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h
Isolates of *Malassezia pachydermatis* (n = 19)														
x	11.4	10.5	14.7	13.5	5.1	4.9	12.1	11	0	0	0	0	12.4	11.8
Min-max	10—15	8—14	8—26	8—26	0—12	0—10	8—13	6—12	0	0	0	0	10—16	8—16
SD	1.51	1.84	5.03	4.97	5.51	5.26	2.13	2	0	0	0	0	2.37	2.44
Efficacy [%]	10	0	30	20	0	0	10	0	0	0	0	0	10	10
***Malassezia pachydermatis* IHEM 19215**														
x	6	6	12	14	6	6	13	10	0	0	0	0	11.5	10.5
Min-max	0—12	0—12	10—14	12—16	0—12	0—12	12—14	8-–2	0	0	0	0	8—15	8—13
SD	6	6	2	2	6	6	1	2	0	0	0	0	3.5	2.5
Efficacy [%]	0	0	0	50	0	0	0	0	0	0	0	0	50	0

x — mean size of inhibition zones in millimetres; min-max — minimum and maximum size of inhibition zones in millimetres
SD — standard deviation; n — number of tested strains

Table 3. Efficacy of alcohols, thymol and copper sulphate against the tested yeasts

Compound/ concentration	Ethanol 70 %		Propanol 70 %		Thymol 0.5 %		Copper sulphate 5 %	
Exposure	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h
Isolates of *Malassezia pachydermatis* (n = 19)								
x	1	0	9	7.7	9.3	8.6	16.5	15.6
Min-max	0—10	0	0—13	0—13	0—16	0—16	11—22	10—20
SD	3.16	0	4.99	5.52	6.96	6.26	2.88	2.84
Efficacy [%]	0	0	0	0	20	10	80	60
***Malassezia pachydermatis* IHEM 19215**								
x	0	0	10	0	3	3	29	27
Min-max	0	0	10	0	0—6	0—6	26—32	24—30
SD	0	0	0	0	3	3	3	3
Efficacy [%]	0	0	0	0	0	0	100	100

x — mean size of inhibition zones in millimetres; min-max — minimum and maximum size of inhibition zones in millimetres
SD — standard deviation; n — number of tested strains

Table 4. Efficacy of quarternary ammonium compounds and chlorhexidine against the tested yeasts

Compound/ concentration	Benzododecine 0.5 %		Carbethopendecine 0.8 %		Chlorhexidine 2 %	
Exposure	72 h	96 h	72 h	96 h	72 h	96 h
Isolates of *Malassezia pachydermatis* (n = 19)						
x	22.7	23.2	14	13.2	16.4	15.8
Min–max	20—26	20—31	11—20	10—18	13—27	12—25
SD	1.95	3.12	3.06	2.66	4.09	3.71
Efficacy [%]	100	100	30	30	60	60
***Malassezia pachydermatis* IHEM 19215**						
x	23	21	15	14	17.5	16.5
Min–max	20—26	18—24	10—20	10—18	15—20	15—18
SD	3	3	5	4	2.5	1.5
Efficacy [%]	100	100	50	50	100	100

x — mean size of inhibition zones in millimetres; min–max — minimum and maximum size of inhibition zones in millimetres
SD — standard deviation; n — number of tested strains

The most frequently used antiseptics belong to the following groups: oxidants (hydrogen peroxides and halides), compounds containing heavy metals (silver nitrate, copper sulphate) and organic compounds (alcohols, phenols, aldehydes, acids, biguanides, organic dyes and detergents) [6].

Information about the antiseptic efficacy against yeasts is scarce. Most authors base their conclusions on the general properties of compounds and consider their antibacterial spectrum to be the decisive factor. However, more frequent is the testing of preparations (disinfectant, cosmetics) composed of several antiseptics.

According to our results, hydrogen peroxide was the most effective substance against *Malassezia pachydermatis* strains. Hydrogen peroxide acts as an oxidant by producing hydroxyl free radicals (•OH) which attack the essential cell components, including lipids, proteins and DNA. It has been proposed that exposed sulphydryl groups and double bonds are particularly targeted [16].

Potassium permanganate in 0.2 % concentration has been recommended for treatment of oozing skin lesions, cleaning of wounds and as a water bath [6]. However, it was ineffective against all strains tested in this concentration.

As it was discovered in this study, 100 % activity was achieved with tincture of iodine and Lugol's iodine. Very good efficacy of 4 % potassium iodide as the component of tincture of iodine against *Candida albicans* has also been reported [23]. Although aqueous or alcoholic (tincture) solutions of iodine have been used for 150 years as antiseptics, they are associated with irritation and excessive staining [16]. Other work claimed that povidone iodine, in concentrations of 0.5—1 %, inhibited the growth of lipid-dependent *Malassezia* species *M. sympodialis* and *M. furfur* [10]. Povidone iodine (iodophor) is also recommended for the therapy of skin mycoses, particularly infections with dermatophytes of the genus *Trichophyton* species and causative agents of *tinea* (*pityriasis*) *versicolor — Malassezia furfur* and *M. globosa* [22]. Our testing of povidone iodine activity against *Malassezia* yeast showed its ineffectiveness. Iodophors are complexes of iodine and a solubilizing agent or carrier, which acts as a reservoir of the active free iodine. Although germicidal activity is maintained, iodophors are considered less active against certain fungi and spores than are tinctures [16].

Commonly used organic acids for skin disinfection are; salicylic, acetic, propionic and benzoic acid. In the treatment of *otitis externa* caused by the yeast *Malassezia* species, 2 % acetic acid and 2 % boric acid were effective. A combination of 0.5 % acetic acid with 3.5 % boric acid was very effective against *Candida albicans* [1]. Salicylic acid was recommended for local treatment of seborrhoeic dermatitis, acne and mycotic diseases of skin [18]. At concentrations up to 3 % it exhibits keratoplastic effect and the effect is keratolytic at concentrations above 10 % [6]. Salicylic, acetic and propionic acid tested in this study were unsuitable and their effect was minimal.

Although several alcohols have been shown to be effective antimicrobials, ethyl alcohol (ethanol, alcohol), isopropyl alcohol (isopropanol, propan-2-ol) and n-propanol (particularly in Europe) are the most widely used. Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), viruses, and fungi but are not sporicidal [14], [16]. Phenolic compounds were considered to be very effective disinfectants for a long time. They seriously damage cell membranes and organelles. Currently they are considered protoplasmic poisons and their use is limited [8]. By the testing of antifungal activity of thyme essential oil, containing approximately 33 % of thymol, very good results against various micromycetes (*Aspergillus* spp., *Alternaria* spp., *Penicillium* spp., *Absidia* spp., *Mucor* spp., *Cladosporium* spp. and others) were obtained [20]. Our results show that the inhibitory activity of thymol against *M. pachydermatis* was negligible.

Solution of copper sulphate pentahydrate, known as bluestone, has been considered an effective fungicide and this fungicidal action was also confirmed in our study. Copper sulphate and chlorous acid in the form of a 1 % solution appeared suitable for the treatment of dermatophytosis in rabbits caused by *Trichophyton mentagrophytes* [11]. In concentrations of 2 % and higher, the solution is effective for therapy of panaricia in sheep [6].

From the group of quaternary ammonium bases, the effectiveness of benzododecine and carbethopendecine were tested. These compounds have been used in practice as a 0.5—1 % solutions for the disinfection of wounds and operation in the field [6]. Benzododecine exhibited excellent (100 %) activity, while the effect of carbethopendecine was insignificant (30 %).

Chlorhexidine in a 2 % concentration is a component of several polycomposite preparations against mycoses. In this concentration, it was effective against 60 % of the strains tested in our study. Topical antiseptic products used for management of canine otitis, generally referred to as ear cleaners, may contain different molecules with antimicrobial activity, e. g., chlorhexidine and ethylene diamine tetra acetic acid-tromethamine (Tris-EDTA). Combinations of these compounds showed an excellent fungicidal activity against *Malassezia* species [11]. 2—4 % chlorhexidine in the form of a shampoo or spray for the treatment of candidosis and malasseziosis, is suggested [19]. The sensitivity testing of *Candida albicans* yeasts to four different disinfectants proved the best efficacy of 0.5 % chlorhexidine. Its combination with 5 % calcium chloride prolonged its action [23]. Also a combination of 2 % chlorhexidine and 2 % azole antibiotic miconazole is recommended for the local therapy of dermatitis caused by yeasts *Malassezia* spp. [17].

ACKNOWLEDGEMENTS

This study was supported by the Slovak Research and Development Agency under the contract APVV-0357-07.

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Received April 30, 2014

FOLIA VETERINARIA, 58, 1: 53—56, 2014

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EXAMPLES

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Black, H., Duganzich, D., 1995: A field evaluation of two vaccines against ovine pneumonic pasteurellosis. *New Zeal. Vet. J.*, 43, 60—63.

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