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BOVINE MYCOTIC MASTITIS
(A Review)

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ABSTRACT

The prevalence of mycotic mastitis is usually very low (1—12 % of all mastitis cases), but sometimes it can occur in epizootic proportions. Fungal infections of the mammary gland are predominantly caused by yeasts of the *Candida* genus. Bovine mastitis due to yeasts and moulds is usually associated with contaminated antibiotic preparations. Yeast intramammary infections are usually self-limiting with spontaneous recovery and the infections usually have no systemic consequences. Amphotericin B, fluconazole, tioconazole, miconazole, Nystatin are used for treatment of mycotic mastitis. Non specific prophylaxis relies on the elimination of all predisposing factors causing mycotic mastitis and the observation of cows treated with antibiotics.

Key words: cattle; fungi; mastitis; milk; yeasts

INTRODUCTION

Since the introduction of antimicrobials in 1945, corthicotherapy in 1950, immuno-suppressive therapy in 1966 morbidity due to fungal infections has increased. Over the last decade mortality and morbidity due to numerous forms of mycoses in humans and animals has increased world-wide at an alarming rate.

Among the more than 72.000 described species of fungi that are widespread in nature (soil, plants, water, air), over 300 are now recognised as real or potential pathogens responsible for mycoses in humans and animals (5).

The word *fungus* is derived from the Latin *fungus*. The Latin was derived from the Greek *sphongirs* or *spongae* (5).

The most important work in the history of mycology was the work of Sabouraud. His medium for the identification of fungi still bears his name. Sabouraud's medium is generally accepted and commonly used (5).

Inflammation of the mammary gland is the most important health problem in bovine dairy herds. The prevalence of mycotic mastitis is usually very low, but sometimes it can occur in epizootic proportions.

Several species of fungi have been reported in many countries as causative agents of mastitis (1—4, 6—8, 10, 13, 17, 19—35). Fungal infections of the mammary gland are predominantly caused by yeast (Table 1). The main genus *is Candida*. Mastitis caused by yeast of the *Cryptococcus* genus and by moulds is rare (10).

Yeasts are micro-organisms which are maybe found on a wide variety of substrates such as soil, plants, water, nectar of flowers, fruits, exsudates of animals. The nomenclature of the yeast isolated from affected patients has changed. Lagenbeck in 1839 first demonstrated a yeast-like fungus in the lesions of thrush. His finding was confirmed by Gruby in 1842. This micro-organism was named *Oidium albicans* by Robin in 1853*, Syringospora robini* by Quiquod in 1868, *Saccharomyces albicans* was named by Rees in 1875. Zopf named the fungus *Monilia albicans* in 1890, and for many years the disease was known as *moniliasis*. Berkhaul renamed the organisms *Candida albicans* in 1923 (*ex* 5, 15). The genus *Candida* was accepted as the official name by the Eighth Botanical Congress in Paris in 1954 (5).

Infections caused by fungi of *Candida* genus have been known in animals for over 140 years. Eberth reported candidiasis of chickens in 1858. In 1932 Gierke reported an outbreak of candidiasis in turkey poults (15). Fleischer in 1930 was probably the first to describe a case of mycotic mastitis (2).

ETIOLOGY

Fungi have been isolated from milk samples with and without symptoms of mastitis. Fungi are not unusual agents

Tab. 1. Fungi isolated from the mammary secretion of lactating cows and buffaloes [according to: 1–3, 6–7, 10–13, 16, 20–22, 24, 26 35]

GENUS	SPECIES
Candida	*C. albicans, C. beigelii, C. brumptii, C. catenulata, C. ciferri, C. freyschusii, C. famata (Torulopsis candida), C. glabrosa, C. globosa, C. guillermondii, C. hemuloni, C. humicola, C. inconspicua, C. ingens, C. intermedia, C. krusei, C. lusitaniae, C. lambica, C. membranaefaciens, C. mogii, C. norvegensis, C. parakrusei, C. parapsilosis, C. pelliculosa, C. rugosa, C. shehate, C. solani, C. sorbosa, C. stellatoidea, C. tenuis, C. kefyr (C. pseudotropicalis), C. tropicalis, C. zeylanoides, C. variabilis, C. valida*
Rhodotorula	*R. glutinis, R. minuta, R. rubra*
Trichosporon	*T. cutaneum (T. beigelii), T. asahii*
Cryptococcus	*C. albidus,C. curvatus, C. flavus, C. laurentii, C. luteolus, C. neoformans*
Aureobasidum	*A. pullulans*
Pichia	*P. ohmeri, P. membranaefaciens, P. farinosa, P. toletana, P. rhodanensis*
Geotrichum	*G. candidum, G. capitatum (Blastoschizomyces capitatus)*
Saccharomyces	*S. fragilis, S. marxianus*
Debaromyces	*D. hansenii*
Hansenula	*H. fabianii, H. holstii, H. polymorpha, H. anomala*
Aspergillus	*A. fumigatus, A. flavus, Aspergillus* sp.
Penicillium	*P. chrysogenum, P. cyclopium (P. aurantiogriseum), Penicillium* sp.
Alternaria	*Alternaria* sp.
Epicoccum	*Epicoccum* sp.
Phoma	*Phoma* sp.

in bovine mastitis and are usually considered as an environmental mastitis due to poor animal hygiene (3, 31). Bovine mycotic mastitis was reported (2, 3, 22, 23) to be responsible for 1—12 % of all mastitis cases. Yeasts predominantly cause bovine mycotic mastitis (Table 1). In our study an occurrence of 9.6 % of yeasts isolated from the mammary secretions of cows with mastitis was registered. The fungi were isolated in a pure culture (21).

The moulds (*Aspergillus, Penicillium, Epicoccum* and *Phoma* genus) were isolated from mastitic secretions of mammary glands in lactating cows and buffaloes in tropical countries (2, 3, 8, 16, 17, 26, 29). Several species of *Aspergillus, Phoma* and *Epicoccum* causing mastitis, produce mycotoxins, as well, are resistant to pasteurisation and with regard to this they are not neutral in terms of human health (2).

THE PATHOGENICITY OF FUNGI

The ability of the fungus to attach to host tissue, the production of proteolytic enzymes (secretory aspartyl proteases – SAP), leucine aminopeptidase and immunomodulatory effects of fungal determinants are the virulence traits of fungi. These virulence factors have been attributed with possible roles in the pathogenesis of candidiasis. Adhesion to tissues was found to be dependent on environmental conditions affecting the fungus (30).

Jensen and Aalbaek (14) described the pathogenicity of yeasts isolated from bovine mastitis secretions in murine models. According to them, yeasts, which killed mice, were *Candida tropicalis* and *Geotrichum capitatum*. The group, including the following species of yeast: *C. kefyr, C. krusei, C. rugorsa,* were classified as having moderate pathogenicity for mice. The yeast of *C. valida* and *C. catenulata* species was classified as having low pathogenicity for mice. The mammary glands of mice inoculated through the teat canal with a suspension containing *Candida albicans* blastospores showed marked neutrophilic infiltration and severe necrosis.

Evidence is gradually accumulating that *Candida* spp. other than *Candida albicans* have pathogenic potential for animals. The predisposing factors are probably equally or more so for them (30).

SOURCE OF THE FUNGI AND PREDISPOSING FACTORS

Predisposing factors to fungal infection of mammary gland are irregular environmental circumstances in cowsheds and, above all, much too high a humidity. Litter is the main habitat of funguses, with especially favourable conditions for their multiplication in old, moist straw and sawdust (22).

The source for yeast can be intra-mammary infusion from a previous antibiotic therapy. The outbreaks usually were associated with contamined antibiotic preparations used for intramammary infusions. These antibiotic combinations are often home-made. The use of antibiotics might have eliminated the

commensal bacteria of the mammary gland and might permit yeast multiplication (2, 6, 10, 13, 17, 23, 25, 31).

According to Kauker (18), yeast mastitis occurs after long term treatment with high doses of penicillin, which lead to a lowering of vitamin-A-levels. The consequences are defects in the serosa of the udder and the facilitation of the invasion of fungi.

The results of antibiotic therapy are: stimulation of the fungus, the possible damage to tissues by the offending antibiotics, the conversion of *Candida* spp. to a more invasive form and the depression of host responses to the infection. Tissue damage by antibiotics may facilitate local invasion by *Candida* spp. and the lowering of resistance of tissues to invasion. Furthermore, *Candida albicans utilises antibiotic tetracycline as a source of nitrogen (2, 15).*

We also concluded in our study (20, 21), that in small dairy farms in the Lublin region (Poland), the development of mastitis due to yeast species sometimes occurred after an intra-mammary infusion of antibiotics. It was found that fungal mastitis appeared mostly after antibiotic treatments (large doses), which was done often without microbiological examinations of milk from the affected quarters or after an infusion of antibiotics that were often home-made.

Elad et al. (6) described potential sources and ways of transmissions of *Candida krusei* as an etiological agent of mastitis. *Candida krusei* was isolated from different sources such as: fluid samples from milking equipment, feed, feed components and faecal samples. *Candida krusei* was isolated from wheat silage and from citrus peel also. Wheat silage was therefore the most probable source of contamination, because cases of mycotic mastitis were diagnosed only when this silage was incorporated in the feed mix.

Lagneau *et al.* (23) compared the percentage of yeast presence of normal milk samples from dairy herds without mastitis with that of normal milk samples collected in a dairy herds with some mastitis cases. The difference was statistically significant. The authors concluded, that healthy animals in dairy herd, where mastitis cases were observed, offer favourable conditions for the multiplication of yeast in milk. This could be result of a serial treatment with antibacterial antibiotics given blindly to all animals.

Yeasts are always present on the skin of the udder and teats, and can enter the teat canal. Teat injuries may predispose the udder to yeast intra-mammary infections. Yeasts of the *Candida* genus were also isolated from teat cups of milking machine and they can be transmitted from cow to cow at milking time. The inappropriate use of instruments such as cannulas, syringes, may predispose the udder to mastitis also. When old needles stuck through stoppers or dusty stoppers are used, the potential risk increases for yeast intra-mammary infections (6, 10, 31).

SYMPTOMS

Yeast intra-mammary infections are usually self-limiting with spontaneous recovery and the infections usually have no systemic consequences.

In some cases the infected cows with acute yeast mastitis show the following signs: fever (40—41 °C), increased heart rate and anorexia. Milk production reduces dramatically. Affected udders are red, hot, swollen, hard and painful and sometimes with severe indurations. Mammary secretions are thick and yellowish or have many flakes, and some contain blood. The California Mastitis Test (CMT) is positive. Mastitic secretions of mammary glands have the characteristic smell of yeast. At times of enzootic mycotic infection of udders, the scent of yeast is evident even in the cowshed and sometimes the owners of animals mention it.

Kitamamura *et al.* (19) described the pathologic findings of chronic mastitis caused by *Candida maltosa* in 5.5-year-old Holstein-Friesian cow. The mastitis was characterised by swelling and induration but involved only one quarter of the udder. Histologically, mammary lesions were divided into two types: extensive suppurative inflammations with necrosis, and giant cell formations. The supramammary lymph node showed follicular lymphoid hyperplasia, sinus histiocytosis with some neutrophilic infiltration, and plasmocytosis in the medullary cord.

Subclinical yeast mastitis is characterised only by CMT, which is positive. Subclinical inflammations of udders as a rule remain a very long time and there is lack of other changes except in terms of an enlarged number of somatic cells and presence of yeast in milk.

Bovine udder aspergillosis at 5-year-old Holstein-Friesian cow was described by Katamoto and Shimada (17). A quarter of the udder was swollen, fevered, firm, hot and with severe induration. Clots were present in the milk and CMT was positive. The cow was anorexic and depressed. Its rectal temperature was 41.2 °C. *Aspergillus fumigatus* in pure culture was isolated from the milk sample.

LABORATORY DIAGNOSIS

Microbiological examinations of the secretions of the mammary gland are necessary for the proper recognition and correct diagnosis of mycotic mastitis. With regard to the ubiquitous occurrence of fungi in the environment, the results of mycological examinations of milk sample can be misleadingly positive. The most convincing evidence of an infection in mammary gland is the presence of fungi in blister milk, gained from the last streams of milk during milking (22).
Yeast colonies grown on blood agar at 37 °C for 24—48 hours may be confused with and mis-identified as staphylococci or micrococci. Colonies of *Candida* spp. generally are opaque, white or yellowish. Colonies of *Cryptocoocus neoformans* initially are pale and pasty becoming honey-brown and mucoid later (10). When a growth of fungi is noticed, the colonies of yeasts should be plated on Sabouraud agar with antibiotics (chloramphenicol, gentamicin) and should be incubated at 37 °C for 48 hours. Genera and species of the yeasts should be identified by the API C AUX system.

TREATMENT

The treatment of yeast inflammations of mammary gland can be undertaken only after the mycological settlement of etiological agent. A lack of ready, antifungal intramammary

preparations causes the treatment of fungal mammary infection to create difficulties. Amphotericin B, fluconazole, tioconazole, miconazole, Nystatin are commonly used for treatment of mycotic mastitis.

Van Damme (34) reported that miconazole was effective on bovine mastitis caused by fungi. Katamoto and Shimada (17) have described the treatment of bovine mastitis caused by *Aspergillus fumigatus*. 100 mg of miconazole (10 ml) was injected into the right external pudendal artery. Miconazole (100 mg) diluted with 50 ml of a saline solution was also infused into the affected udder. The same therapy was continued for three successive days. *Aspergillus fumigatus* was detected for three days after the initial treatment but then disappeared. The treatment was effective.

The therapeutic use of tioconazole was assessed in terms of udder tolerance, minimal inhibitory concentration (MIC) and concentration of tioconazole in milk after intramammary applications. None of the treated cows showed clinical symptoms. Applications of tioconazole caused only slight udder irritation when compared with commonly used antimycotic drugs. Tioconazole elicited the highest *in vitro* activity against isolates of *Candida lusitaniae* and *C. kefyr* and the lowest activity against *Pichia farinosa* (9).

Eight cows with clinical fungal mastitis were treated with Betadine (povidone iodine) by intramammary infusion for five days. The treatment was completely effective in six cows but there was no effect in two cows (32).

The use of Lamisil (terbinafine) in the treatment of some cases of mycotic mastitis in cows in Egypt was very effective, resulting in a complete recovery (16).

Mycotic mastitis in buffalo caused by *Candida tropicalis* was treated using Nystatin. The drug was well tolerated and showed a positive clinical response. Examination of milk from the buffalo fourteen days after treatment failed to reveal the presence of *C. tropicalis* (27).

The result of our investigations showed, that best effects of treatment are obtained after two (over a forty-eight hours) intramammary infusions of Nystatin dissolved in distilled water or in a 5% solution of glucose (22).

The murine model of mycotic mastitis was used also to study the efficacy of amphotericin B and fluconazole. It is concluded that amphotericin B is more effective in the prevention of murine mycotic mastitis than fluconazole and the murine mycotic mastitis model may be useful as an animal model for antifungal chemotherapy studies (11, 12).

PROPHYLAXIS

The specific prophylaxis of mammary gland fungal infections does not exist because immunization against mycotic mastitis has, as yet, no clinical validity in the prevention of fungal infection of the mammary gland in cows. The immune response of the mammary gland to fungal infection, antigens eliciting this response and, ultimately, immunity are unknown. Non-specific prophylaxis relies on the elimination of all predisposing factors causing mycotic mastitis and the observation of cows treated with antibiotics.

Therefore, it should be concluded that fungal mastitis will become an increasing problem due to the widespread use of antibiotics in mastitis therapy.

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FORESTOMACH ABSORPTION OF AMINO ACIDS AND PEPTIDES (A Review)

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ABSTRACT

A primary concern of animal nutritionists is the need to understand what the capacity for α-amino acid absorption is, so that diets can be formulated to provide adequate, but not excessive, amino acids for a given production state. A small peptides represent a source of free amino acids. Data from this review indicate the interest of recent research in identifying the capacity for forestomach absorption of amino acids and peptides.

Key words: absorption; amino acid; forestomach; peptide; ruminant

INTRODUCTION

The literature is replete with characterizations of free and peptide-bound amino acid transport systems that are present in by laboratory animals and humans. In comparison, little research has been conducted to identify the presumably analogous transport system in farm animal species. Given the economic importance of these species, and the high rates of growth and protein production currently demanded by producers, the lack of knowledge regarding specific farm animal transporter physiology may limit our ability to formulate diets and design feeding strategies that optimize protein synthesis and retention.

These conclusions also result from the experimental work which was carried out from 1987 to 2002 at the Department of Pathophysiology in the University of Veterinary Medicine in Košice.

RUMINAL AMINO ACID CONCENTRATION

The proteolysis of dietary protein, microbial synthesis, amino acid catabolism, microbial lysis and the escape of amino acids from the rumen determine the concentration of free amino acids in ruminal fluid.

In general, ruminal free amino acid concentrations increase to the highest level one hour postfeeding and then rapidly return to the prefeeding levels within two to three hours after feeding (31). The transient accumulation of free amino acid depends on the nature of the dietary protein N source, ration size, feeding frequency (29) and the rate of bacterial uptake of amino acids (12). The results of Ling and Armstead (19) and Armstead and Ling (1) have shown that several species of ruminal bacteria prefer free amino acids rather than peptides, but they have also suggested that these effects may be influenced by dietary composition, which has an effect on the microbial species present in the rumen (4). Martin (21) has described several active transport mechanisms for amino acid in the ruminal bacteria. The amino acids are incorporated into microbial protein or degraded into α-keto acids and ammonia or passed from the rumen to successive parts of the digestive tract. The ileum region of sheep and cattle has been identified as possessing the greatest potential for free amino acid absorption (16, 27, 11).

Little is known about the ability of the ruminant forestomach epithelium to absorb amino acids.

FORESTOMACH AMINO ACID ABSORPTION

The study of transport systems in forestomach tissues (rumen, reticulum and omasum) is complicated by the structure of their keratinized squamous epithelia. Starting proximal to the forestomach liquor, and ending adjacent to the basement

membrane, the four strata of epithelial cells that make up these tissues have been classified as the *basale, spinosum, granulosum* and *corneum.* The number of cell layers that comprise the whole epithelia can range from eight to less than thirty. Given the technical difficulties in performing kinetic characterization for such a complex tissue, and the classic hypothesis that the forestomach is responsible for little, if any, net absorption of amino acids, little research has been conducted to identify the capacity for forestomach absorption of amino acids or peptides. Consequently, mechanisms capable of α-amino nitrogen absorption in forestomach tissues are poorly characterized.

Given that pre-feeding levels of free amino acid concetrations in strained ruminal fluid of 0.12—0.15 $mg.dl^{-1}$ and post-feeding concentrations of 0.72—6 $mg.dl^{-1}$ have been reported in the rumen fluid of sheep fed common diets (22), it is likely that amino acid transporters would be of the high-affinity type. In cells and membranes exposed to high substrate concentrations, the expression of low-affinity, high-capacity transporters is typical, whereas those normally exposed to low substrate concentrations often express high-affinity, low-capacity transporters.

The amount of amino acids absorbed across the rumen wall represents about 10 % of the total nitrogen which is absorbed into the blood from the rumen (13). Bochroder *et al.* (2) have studied the transmucosal fluxes of the amino acids lysine, histidine and arginine across the colon of the equine. Results show that when the amino acid concentrations in the mucosal solution were in the physiological range (2.8—3.0 $mmol.l^{-1}$) no transport to the serosal side of the tissue was found. When the concentrations were raised tenfold, less than 2 % of the mucosal amino acid pool was recovered in the serosal solution.

Matthews and Webb (23), studying the transepithelial passage of methionine (from 0.66 to 10 $mmol.l^{-1}$) across sheep ruminal and omasal epithelial sheets, have concluded that absorption was interpreted to mean that absorption was not saturable. These results indicate that omasal epithelial tissue possesses a greater ability to absorb free amino acids than ruminal epithelial tissue. In another *in vitro* study (15) it was found that free amino acid absorption across the omasal epithelium was minimal.

In retrospect, however, the fact that methionine, arginine and glycine inhibited histidine transfer across ruminal epithelium indicates that histidine transfer may have been at least partially mediated by transport proteins (17).

Additional evidence that cationic amino acid absorption from forestomach liquor is mediated comes from the observations that the flux of lysine and arginine across ruminal tissue sheet is saturable from 0.3 to 30 $mmol.l^{-1}$ (9) and leucine in an incubation medium has inhibitory effect on the lysine flux across isolated ruminal tissue of sheep (7).

In agreement with these observations Rémond *et al.* (28) have reported that ruminal net flux of lysine decreased in a quadratic manner as the level of amino acid dosed increased *in vivo* conditions.

McCollum *et al.* (25) have studied the absorption of 2-hydroxy-4-(methylthio) butanoic acid (HMB; a source of L-methionine used in nonruminants and ruminants) across ovine ruminal and omasal epithelia using parabiotic chambers throughout a sixty minute incubation. After sixty minutes of incubation, the accumulation of HMB in the ruminal epithelia increased linearly as the substrate concentration increased in the mucosal buffer. In an experiment with omasal tissue, as the concentration of HMB in the mucosal buffers increased, there was a quadratic increase in the appearance of HMB in the mucosal buffer of the omasal epithelium, indicating some saturation of the system. It is likely that mediated transport accounts for at least a portion of the absorption of HMB in the omasum. Other mechanisms (e.g., diffusion and/or paracellular absorption) are responsible for the balance of absorption. The enzymes involved in the conversion of HMB to 2-keto-4-(methylthio) butanoic acid were found in ruminal and omasal epithelia. These results indicate that HMB can be used as a source of L-methionine.

Results of experiments indicate that amino acid absorption across the ruminal epithelium is dependent on several factors.

Leng *et al.* (18) have found that amino acid transfer across the ovine rumen epithelium is dependent on the α-amino nitrogen concentration in the rumen. Faixová and Várady (8) have described that both dorsal and ventral rumen epithelium can absorb ^{14}C-lysine to the same extent. Later, measurement of the transepithelial passage of glutamic acid, lysine and arginine across the ruminal epithelial sheets of adult sheep and lamb have indicated that there are differences in amino acid absorption across the rumen in lamb on a milk diet when the rumen is not fully morphologically developed as opposed to that of adult sheep (6). The ruminal net flux of L-leucine, L-alanine and L-lysine from the washed closed rumen decreased in proportion to the fall of amino acid concentration (30).

RUMINAL PEPTIDES CONCENTRATION

Significant amounts of peptides have been detected in the rumen early after feeding (1—2 hours) and do not exceed 8 $mmol.l^{-1}$ as the rate of production exceeds uptake and metabolism by microorganisms (14). During the rest of the feeding cycle, peptide amino acid concentrations are below 1 $mmol.l^{-1}$ (32).

The extent to which peptides accumulate has also been shown to be dependent on the peptide source and feeding frequency (33), and in more recent studies, the interaction between feed protein and physical form of the dietary carbohydrate has been studied.

Broderick and Wallace (3) have reported that little peptide remained undegraded three hours after feeding but recent work by Wallace *et al.* (32) has identified the presence of degradation-resistant peptides in the ruminal fluid of sheep six hours after feeding, which are characterized by containing the amino acids aspartate, glycine, and proline.

Rapidly degraded proteins represent an important source of peptides in the ruminal fluid. But little is known about the ability of the ruminal forestomach epithelium to absorb this product of dietary degradation.

FORESTOMACH ABSORPTION OF PEPTIDES

In vitro studies with isolated epithelium suggested that ruminal epithelium is permeable to free (30) and peptide-bound (methionylglycine) amino acid (23).

Dipeptide carnosine (β-alanylhistidine) has been used as the initial substrate in several experiments to investigate the potential for dipeptide absorption by forestomach epithelia because of its relative resistance to hydrolysis by dipeptidases and its recognition by characterized peptide transport proteins (10).

The results of Matthews and Webb (23) have indicated that the amount of carnosine that passed through ruminal and omasal tissue, was not saturable in observed concentrations. That histidine was not detectable above background levels indicates that carnosine had spassed through both tissues without hydrolysis or has been completely degraded by the tissue. The omasal tissue of sheep seems to possess a greater ability to absorb carnosine than did ruminal epithelium.

The expression of sheep omasal mRNA in oocytes (24) results in H^+-dependent Gly-Sar uptake that is consistent with Pep T_1 transport characteristics. Additional work has confirmed these findings and demonstrated that sheep omasal peptide transport protein(s) display differential affinities for di-, tri- and tetra-peptides (26).

Recently in the work of Rémond *et al.* (28) it has been demonstrated that increasing level of carnosine injected in the rumen and in the omasum leads to a linear increase in carnosine net release in the right ruminal and the left gastric veins, respectively. But the absorption capacity towards carnosine seems to be low *in vitro.*

In concert with these observations Martens *et al.* (20) have reported that flux rates of D-phenylalanyl-l-alanine) by isolated preparations of rumen and omasum tissue of sheep by using the Ussing-chamber method and isolated ruminal cells represent passive and possible probably paracellular diffusion and are not of nutritional importance.

Measurement of the transepithelial passage of free amino acids and peptide carnosine across sheep ruminal epithelium has indicated that amino acid could transfer more easily through the rumen wall in lower initial concentrations that the dipeptide carnosine (5).

CONCLUSION

The ruminant forestomach seems to have the capacity to absorb free amino acid and peptides. However, the permeability of the forestomach to free amino acids and carnosine seems low. Therefore, under normal feeding conditions, the forestomach probably is not a major site of free amino acid absorption, but, with heavy oral supplementation of free amino acids, this absorption may become significant.

If future research confirms the potential of forestomach tissue to absorb peptide-bound amino acids, then the practice of supplementing the amino acid requirements of ruminants with proteins designed to be absorbed by the forestomach epithelia may become an integral component of ruminant diet formulation.

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CHANGES DETECTED IN THE ORGANS OF QUAILS WITH EXPERIMENTALLY INDUCED HYPERTHYROIDISM

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SUMMARY

In the present study, pathological changes in the organs of quails with experimentally-induced hyperthyroidism were examined. For this purpose, 30 four-week-old male Japanese quails were used. Twenty of these were separated as the experimental group, and the remaining 10 were used as controls. The quails in the control group were fed with normal quail feed, while the quails in the experimental group were given 0.4 mg.100 g^{-1} L-thyroxin in their feed for a period of 21 days. Blood samples were collected from both groups on the 21st day of the experiment, and plasma T_4 levels were determined. Necropsy was performed on the quails of both groups which were euthanized with ether at the end of the 21st day. A varied enlargement of the hearts and the width of the left ventricle of the 10 quails in the experimental group were observed. Microscopically, a thickness in the muscle fibres and an increase in the number of muscle cell nuclei were observed. Moreover, it was observed that the follicular epithelial cells of the thyroid gland were atrophied. The changes in body weight and the weight loss in the testicles of all quails in both groups were determined to be statistically insignificant. The increase in plasma T_4 levels was found to be significant ($P < 0.01$).

Key words: hyperthyroidism; quails; pathological findings

INTRODUCTION

Hyperthyroidism is an endocrine disorder caused by the increase of thyroid hormones in the blood (20, 15, 8, 7).

This disease may occur spontaneously as a result of the excessive production and secretion of T_3 and T_4 hormones by the thyroid gland (15, 7, 8). This case generally occurs due to congenital disorders of the thyroid and occasionally due to disorders of hypothalamus and hypophysis (20, 15, 8). Although hyperthyroidism can occur as a result of acute destruction of thyroid tissue theoretically, no case of hyperthyroidism caused by this reason has been reported in veterinary medicine yet (7). Apart from these, hyperthyroidism can be caused by giving parenteral administration of thyroid hormones (T_3-T_4). Sometimes, this is administered experimentally, whereas it can be caused by giving high doses of T_3-T_4 hormones without control especially for the treatment of hypothyroidism (1, 2, 12, 14, 16, 17, 9, 24, 10). As a result, hyperthyroidism, detected in animals, is mostly dependent on congenital and chronic disorders in one or two lobes of the thyroid gland (18, 3, 23, 7).

This disease may occur in both human and all animal species. Among animals it is mainly encountered in cats. In particular it is one of the most important diseases of middle-aged and older cats (18, 3, 20, 23, 4, 7). Moreover, hyperthyroidism has also been determined in dogs (1, 16, 9). It is sufficient to determine only serum T_4 concentrations in cases of clinically uncertain hyperthyroidism (15, 4, 7, 8).

In cases of hyperthyroidism, important clinical disorders such as tachycardia, increase in pulsation rate and dyspnoea, and abnormalities in ECG have been determined in humans and cats (18, 3, 23, 15, 4, 7, 8). Moreover, some symptoms such as excessive excitability, sensibility to heat, perspiration, weight loss, nervousness and excessive lethargy have also been observed in these patients (15, 7, 8).

It has been stated that, in the cases of hyperthyroidism, blood T_4 level increases, and the levels of triglyceride, cholesterol, and phospholipids in the blood and liver decrease due to the increased usage of lipids as an energy source (1, 6, 15, 7, 8).

It has been stated that in the case of spontaneous hyperthyroidism, whether in humans or in animals, the size of

the thyroid gland becomes two or three times larger than its normal size, the epithelial cells which form the follicles were extremely hyperplasied and they were about to invade the follicle lumina (15, 7, 8). Furthermore, it has been noted that, in some cases of hyperthyroidism, which were induced by giving thyroid hormones with feed or parenterally, the size of the thyroid gland did not change (2, 5, 16, 9, 24, 7). We could not find any research, concerning the pathological changes in the organs of animals other than cats, especially not in avian species with hyperthyroidism. Therefore, in the present study, we aimed to investigate the clinical changes, macroscopical and microscopical changes in the organs of quails with experimentally-induced hyperthyroidism.

MATERIALS AND METHODS

In the present study, a total of 30 four-week-old male Japanese quails (*Coturnix coturnix Japonica*) were used. The quails were divided into two groups, 10 as control and 20 as experimental. While the control group quails were fed with normal quail feed from the Erişler Food firm for 21 days, the birds in the experimental group were given 0.4 mg.100 g^{-1} L-thyroxin in their feed in order to induce hyperthyroidism within the same period. Water and feed were given *ad libitum* to both groups.

Blood samples were taken from *v. cutanea ulnaris* of both control and experimental quails into heparinized tubes, and the plasma was separated by centrifuging the blood at 3500 rpm for 15 minutes. The plasma samples were kept at –20 °C until they were used to determine T_4 levels. Plasma T_4 levels were determined by the radioimmunoassay (RIA) method (19).

On the last day of the study, after the blood samples were taken from all the quails in both groups, the quails were euthanized with sulphuric ether. After their body weights were recorded, necropsies were performed systematically. The weights of testicles were also determined during the necropsies. Later on, pieces of an appropriate size of were taken from all of the organs and dipped into Bouins' solution for fixation. The organ samples which were kept in a fixation solution for 4 days were routinely processed and embedded in paraffin blocks (11). Sections (5—7 μm) prepared from these blocks were stained with haematoxylin-eosin (H-E) and examined under a light microscope. In order to be able to make a quantitative analysis of the testicles of the quails in both the experimental and control groups, the testicle slides prepared from both the experimental and control groups of quails were examined by a micrometric ocular. In each slide, the diameters of 10 different seminiferous tubules in 10 different microscopical areas were measured in 10 × magnification. The result was divided by 100, in order to calculate the mean size of a tubule.

The means of all data, standard deviation of the means and the significance of differences between groups were determined by the Student's *t*-test.

RESULTS

It was observed, especially in the last two weeks of the study, that the quails in the experimental group were sensitive to sound and reacted excessively to humans.

Macroscopical Findings:

The subcutaneous and abdominal adipose tissue of the quails in the experimental group was less than that of the control group. Thyroid glands, livers, kidneys, and intestines of the experimental group of quails were observed to be congested. A slight roundness was noticed in the apex of the hearts. Moreover, a slight thickness was detected in the wall of the left ventricle, and the cut surface was smooth and dry.

Microscopical Findings:

Heart: In some areas, many erythrocytes were observed among the muscle fibres. In these areas a thickness in muscle fibers and an increase in the number of nuclei of the cells was observed. The nuclei were more rounded and polygonal in shape (Fig. 1).

Liver: In all of the liver slides that belong to the quails in the experimental group the veins and some sinusoidal spaces were filled with erythrocytes.

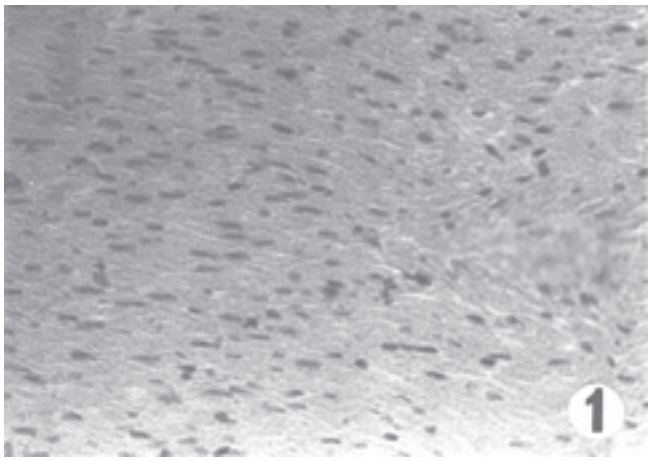

Fig. 1. Increase in the number of muscle-cells (H-E; 400 ×)

Table 1. The mean of T_4, body and testicle weights, standart errors (Sx) and the difference between the groups

	Control group			Experimental group		
	n	$\bar{x}$	Sx	n	$\bar{x}$	Sx
T_4 (mg.ds^{-1})	10	0.76	0.11	20	5.98**	0.36
Body weight (g)	10	163.5	3.36	20	165.14	3.85
Testicle weight(g)	10	5.98	0.74	20	4.80*	0.52

* — P<0.01; ** — <0.01

Thyroid: The veins seemed to be hyperaemic, the colloid in the follicles stained light pink, and some follicles were empty (Fig. 2). Most of the follicles which the thyroid gland consists of were lined with squamous epithelial cells whereas some follicles were lined with cuboidal epithelial cells (Fig. 3).

Testicles: Although some vacuolizations were determined in the germinal epithelium of the seminiferous tubules, spermatogonia were present in most of the tubuli. The mean of the diameters of the seminiferous tubules of the control group was found to be 140.3 mm while this was only 131.6 mm in the quails of the experimental group.

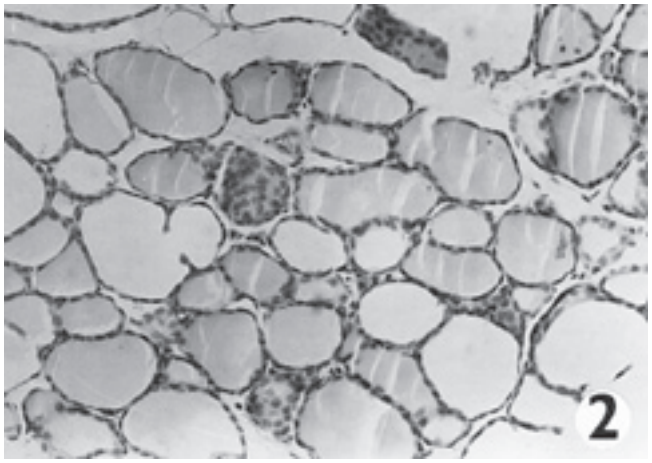

Fig. 2. Colloid deletion or absence of colloid in follicles (H-E; 200 ×)

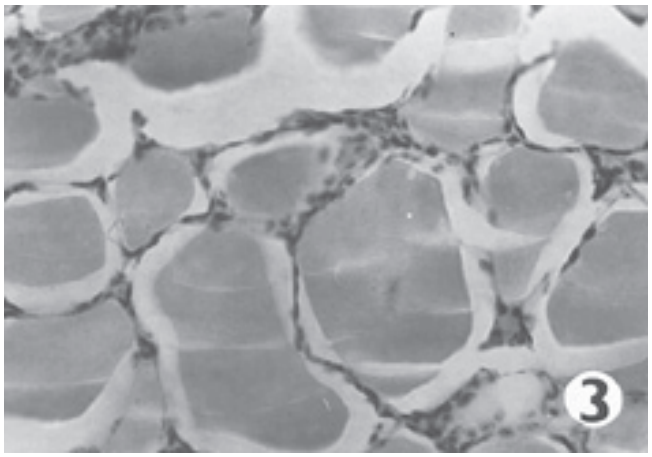

Fig. 3. General appearance of thyroid follicles (H-E; 400 ×)

The mean values of plasma T_4 levels, body and testicle weights of the experimental and the control group quails, standard deviation of the means and the statistical; significance of the differences between the groups are presented in Table 1. A statistically significant increase was observed in the plasma T_4 levels of the experimental group of quails compared to the control group ($P < 0.001$).

A slight increase was determined in the body weight of the hyperthyroid group of quails compared to the control group, but this increase was found to be statistically insignificant (Table 1).

Furthermore, a slight decrease, which was found insignificant statistically, was determined in the testicle weight of the experimental group of quails compared to the control group.

DISCUSSION

The most important effects of thyroid hormones are to increase the oxidative events in the body by stimulating the consumption of oxygen and to increase the metabolism. This effect is confirmed especially by the increase in the activity of the oxidative enzymes in mitochondriae (6, 15, 8). Body weight decreases due to the increase in the catabolism of body proteins and storage lipids. Such animals become nervous easily (6, 15, 4, 7, 8). It has been stated in the studies which were carried out to determine the changes in the body weight of avian species with hyperthyroidism that body weight did not change in the opening phase (the first 2—2.5 weeks) whereas the body weight of the hyperthyroidism group of quails decreased in the course of time (2, 12, 5, 17). It has also been noted in these studies that there is a correlation between the change of body weight and the doses of the drugs which were used to induce hyperthyroidism, and as the dose of the drug increases, the decrease in body weight accelerates.

In the present study, although a slight increase, which was in contrast with the results of some studies (2, 12, 5, 17), was observed at the end of the 3rd week of the study, this increase was found not to be significant statistically. This case can be explained by the low dose of L-thyroxine which was used to induce hyperthyroidism, and by the shortness of the application and trial period.

It has been postulated that the cases of excessive nervousness and sensibility to heat in animals with hyperthyroidism occur in the advanced stages (20, 15, 4, 7, 8). In the present study they were observed clearly in the experimental group of quails especially at the last 2—3 weeks of the study. In this period, it was observed that the quails in the experimental group were sensitive to any kind of sound and that they became aggressive under other stimulations.

In the case of spontaneous hyperthyroidism in both humans and animals, an excessive hyperplasia occurs in the follicle epithelia of the thyroid gland and as a result of this, the thyroid gland enlarges (15, 24, 7, 8). This disorder, which is commonly known as Goitre, is the best known type of hyperthyroidism.

These cases occur either as a result of congenital thyroid disorders or acute destructions of thyroid tissue in the *post partum* period (after birth). Sometimes the disorders of hypophysis and hypothalamus may cause hyperthyroidism (15, 7, 8). Especially in experimental studies, the levels of thyroid hormones (T_3-T_4) in blood increase after the by administration of parenteral thyroid

hormones (1, 2, 5, 9, 24). The hypophysis and hypothalamus are stimulated by negative feed-back mechanisms, and the secretion of Thyroid Stimulant Hormone (TSH) from the hypophysis is suppressed and the secretion of hormone ceases in thyroid gland epithelial cells. And the hormone-secreting, cuboidal, epithelial cells are transformed into simple squamous epithelium in the resting period after hormone secretion (3, 16, 5, 9).

Thus, in such cases of hyperthyroidism it has been observed that the size of thyroid gland did not change, and by microscopical examination it was observed that the epithelial cells which form the thyroid follicles were flattened and the amount of colloid had decreased in some follicles (13, 5, 16, 9, 7).

In the present study, hyperthyroidism was formed by giving L-thyroxine for three weeks. Therefore, it was observed that, as the hormone secretion ceased in the follicular epithelial cells of the thyroid gland, follicular epithelial cells of the experimental group of quails were found to be flattened, and in some follicles a decrease was observed in the amount and density of the colloid. Our findings are in accordance with the results of the relevant literature (5, 16, 7).

The increase of thyroid hormones increases lipolysis in adipose tissues, thus the lipid storage of the body and lipid level in blood decreases, and the levels of cholesterol, phospholipid and triglyceride in blood also decrease (14, 1, 22, 6, 15, 10, 7, 8).

In the present study, because of economical reasons, serum fatty acid, cholesterol and triglyceride levels could not be determined. Furthermore, at the necropsy both subcutaneous and mesenterial adipose tissue, which are known as lipid storage sites, have not been observed in the experimental group of quails. In contrast, a prominent amount of adipose tissue was observed in these regions in the quails of the control group. Moreover, in microscopical examinations, any disorder concerned with fattening was determined in the liver. Although we have no data concerning the serum cholesterol, triglyceride, and fatty acid levels, we can suggest that our necropsy findings support the findings about this fact, as stated above (14, 1, 22, 6, 15, 7, 8).

It has been said that, myocardial hypercontractility, which is a characteristic of hyperthyroidism, occurs in cats with experimentally-induced hyperthyroidism and in humans with thyrotoxicosis (18, 3, 23, 4, 7). Moreover, in the necropsy of cats with hyperthyroidism, a dilatation has been determined in the left ventricle (18, 3, 23).

In the studies carried out on humans, it has been asserted that ventricular hypertrophy was observed at the necropsy of more than 50 % of the patients with hyperthyroidism. Furthermore, it has been stated that hypertrophy in the heart occurs as a result of a long term thyroid hormone application in different species (15, 7, 8).

In the present study, clinical symptoms concerning the heart were not examined. However, in the necropsies, a slight expansion was observed in the heart of 60 % of the quails in the experimental group, the sections of the lumina of the left ventricles were observed to be narrowed and the walls of the left ventricles were found to be thickened. This hypertrophy, which occurs as a result of muscle-cell hyperplasia, was also determined microscopically.

Our findings are partially in accordance with the relevant literatures (18, 3, 23). There are different mechanisms which explain the hypertrophy in the heart, in hyperthyroidism cases. In one of these mechanisms, it has been stated that an increase in myocardial contractions occurs to compensate for the increased tissue perfusion depending on the increase in body metabolism, and the pulsations of the heart increase, and all of these lead to cardiac hypertrophy (18, 3). However, it has also been stated that a hypertrophy in the heart muscle dependent on hyperthyroidism occurs as a result of the increase in protein synthesis and oxygen consumption by the heart muscle (21).

It has been stated that the excess of thyroid hormones causes impotency (8, 10). In the present study no indications of impotency could be observed in the microscopical examinations of the testicles. Moreover the testicle weight of the experimental group quails was found to be less than that of the control group, but this decrease was found to be statistically insignificant..

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THE ACETYLCHOLINESTERASE (ACHE)-POSITIVE COMPONENTS OF PALATINE TONSILS IN RABBITS AND CATS

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ABSTRACT

The occurrence of AChE-positive components of the palatine tonsils in rabbits and cats was investigated by the direct thiocholine method. The largest density of AChE-positive nerve profiles was found in the marginal, i.e. basal and lateral parts of the tonsils, mainly in the form of characteristic periarterial nerve plexuses of different density. Fine nerve fibres line the wall of small arterial vessels which pass for a certain distance in the extrafollicular lymphoid tissue and in the marginal layers of follicles. Penetration of AChE-positive nerve fibres in the centres of follicles were not observed. It is concluded, that all the found differences are usually concerned with the density and arrangement of nerve fibres in different structural part of palatine tonsils. Of note was the apparent non-neural AChE-positive area of lymphoepithelial layer in rabbits.

Key words: AChE-positive components; cat; palatine tonsil; rabbit

INTRODUCTION

The *tonsila palatina* as a part of the mucosa associated lymphoid tissue (MALT) is situated in the *isthmus faucium*. Their functions are connected with the immune mechanism of the organism, including the protection of the respiratory and digestive system.

It is known, that the central nervous system by means of the endocrine and autonomic nervous system also influence the function of lymphoid organs. The presence of autonomic nerve fibres in the parenchyma of lymphoid organs establish an anatomical link between the brain and immune system for translating central neural processing into chemical signals that can influence specific functions of the cellular elements of the immune system (2, 5, 4, 3).

The palatine tonsils belong among the immune organs which develop early. The simplest form of palatine tonsils is in the rabbit and the cat. The free surface and crypts are covered with a stratified squamous epithelium. The lumen of the crypts has no branching and freely communicates with the oral cavity. The lymphatic substance underlying the epithelium is separated by capsulae from the wall of the pharynx. *Capsula fibrosa* is limited only to the base or side of the organ. So the tonsils are peripheral lymphoid organs whose structure is convenient for antigen reception, and they are able to stimulate the whole lymphoid system.

In the literature there are numerous data about the innervation of palatine tonsils in humans (10, 13, 12, 7, 8). In the present study, we have investigated the occurrence of neural and non-neural AChE-positive components of the palatine tonsils in rabbits and cats, as representatives of laboratory animal species.

MATERIAL AND METHODS

Clinically healthy adult animals of both sexes were used in the study. The *tonsila palatina* of twenty cats (from quarantine asylum, 1.8—2.5 kg) and twenty-five rabbits (Chinchilla, 2.5—3.5 kg) were examined. The animals were kept in the Central Animal Husbandry under veterinary care. All animals were anaesthetized with thiopental (50—60 mg.kg^{-1} i.p.). AChE-positive nerve profiles were demonstrated by means of the direct thiocholine method of cytochemical evidence of AChE (1). The investigated excisions were fixed in 4 % paraformaldehyde (pH 7.4) at 4 °C for two to four hours. Sections of 16—20 μm were cut on freezing microtome, incubated in acetylcholinesterase medium (pH 5.5), for two to four hours at 37 °C. Individual

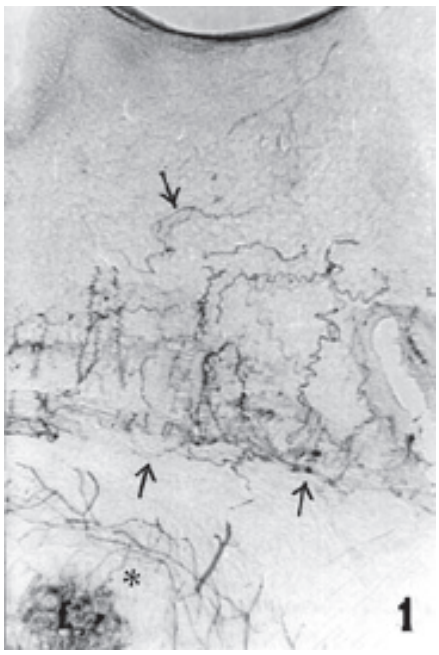

Fig. 1. Abudantly represented AChE-positive nerve fibres in the basal parts of the palatine tonsil in the cat *(arrows).* They lie partly loosely and partly in the form of plexiform periarterial aggregations. The individual nerve profiles *(asterisk)* sometimes also extend in the marginal part of follicles. Magn. × 125

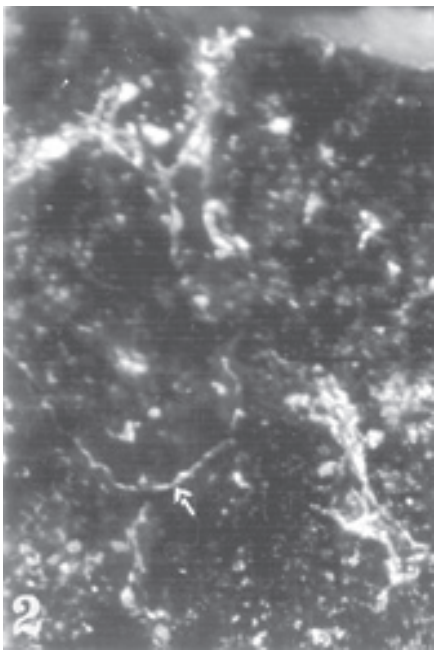

Fig. 2. AChE-positive periarteriolar and individual nerve fibres in the basal compartment of the palatine tonsil in the rabbit *(arrows).* Magn. × 125

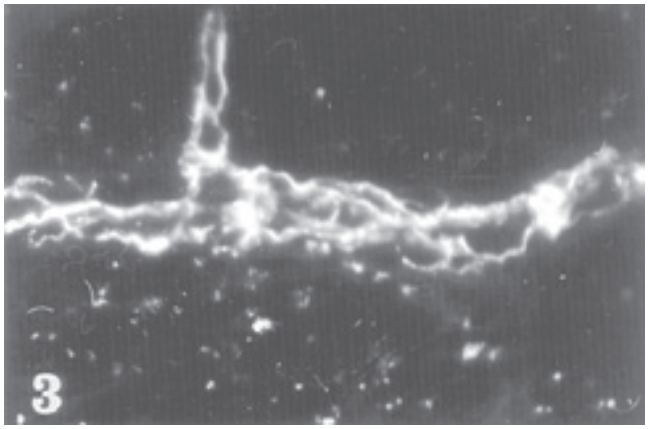

Fig. 3. Partial deposits of the AChE-positive reaction product in the wall of the subfollicular vein *(arrow)* of the palatine tonsil in the cat. Magn. × 250

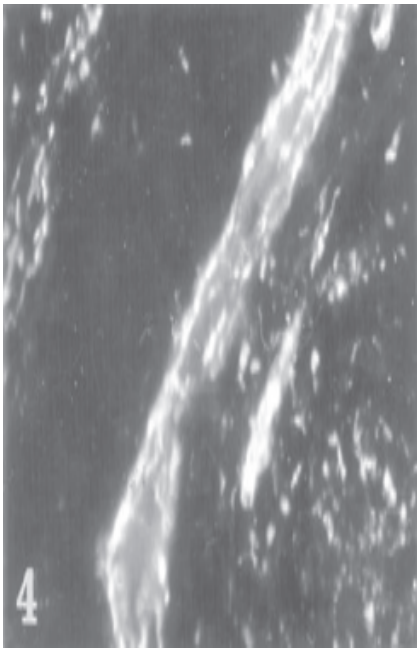

Fig. 4. Linear deposits of nonneural AChE-positive coloured reaction product in the lymphoid follicle of the palatine tonsil in rabbits. Magn. × 500

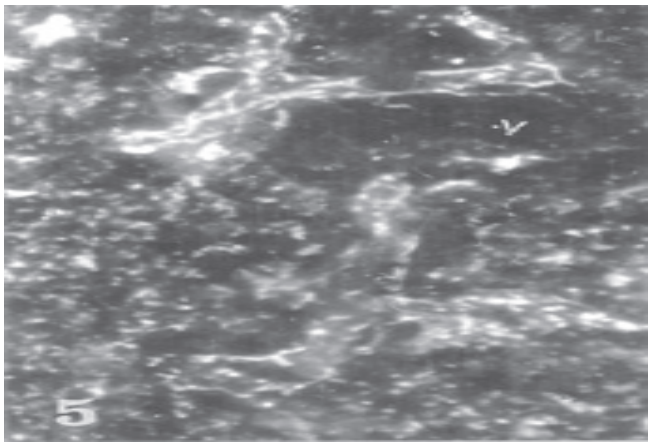

Fig. 5. Pronounced focal nonneuronal AChE-positivity in the superficial lymphoepithelial layer *(arrows)* of the palatine tonsil in the rabbit. Magn. × 125

sections were mounted on glass slides. Control sections were carried out as follows: 1) incubation in a medium free of iso–OMPA (as a selective inhibitor of nonacetylcholinesterases); 2) incubation in a medium containg butyrylthiocholine iodide and BW 284c51 (a selective inhibitor of true cholinesterase). Both the microscopic examination and photographic documentation were performed using Jenalumar 2 (Zeiss, Jena).

RESULTS

The largest density of AChE-positive nerve profiles was found in the adventitia of arterial branches in the *capsula fibrosa* and peritonsilar connective tissue of tonsils in both species of animals which were examined. The profiles had a shape of characteristic plexiform aggregations of variable density. Relatively numerous delicate AChE-positive nerve fibres containing a high amount of a coloured reaction product were recorded in the marginal, i.e. basal and lateral parts of tonsils (Figs. 1, 2). AChE-positive innervation of veins and venules was infrequent and partial (Fig. 3). Nerve fibres accompanied by small arterial vessels in subepithelial layers were not found.

In cats AChE-positive nerve fibres dominate in the interfollicular septa, which sometimes protrude into the basal layers of epithelium. Very delicate nerve fibres were also present in close contact with small arteriolar branches which pass into the extra-follicular lymphoid tissue and for a short distance also into the marginal layers of the follicles themselves. Numerous fine AChE-positive nerve fibres were found only in the marginal zones of follicles in cats.

In rabbits, only fine solitary nerve fibres lie predominantly beside or around lymphoid follicles and they were not observed inside the marginal zones. Penetration of AChE-positive nerve fibres in the centre of follicles (B-dependent area) was not observed.

AChE-positive deposits of the coloured reaction products were also present in the non-neural structures in lymphoid follicles. In follicles they occur as granular and amorphous deposits of a variable density masking the structure of central areas. Furthermore, there were linear forms, which by their appearence and course resemble a reticular skeleton (Fig. 4).

A pronouced focal non-neural AChE-positivity in the superficial lymphoepithelial layer was recorded only in rabbits (Fig. 5), while non-neural AChE-positivity of this layer was not observed. In neither case has this phenomen been observed in cats.

DISCUSSION

Palatine tonsils, like other lymph nodes, are not strongly innervated. The largest density of AChE-positive periarterial nerve profiles was found in the fibrous septum and semicapsule of tonsils and interfollicular septa in both animal species examined, which is in agreement with previous studies (10, 12, 7) in humans.

In cats, we observed numerous AChE-positive nerve fibres travelled in the marginal parts of follicles, whereas in man and rabbits only solitary nerve fibres with small amount of coloured reaction product were found (7). Similarly results were reported by other authors (10, 12, 9), who showed that peptidergic nerve profiles are present in marginal zones whereas germinal centres were devoid of these fibres.

Our finding that in palatine tonsils, as well as in other lymphoid organs, thin nerve branches and fibres run outside of the aggregated lymphoid follicles and not enter inside them, confirm the findings of others (2, 5, 6). They are present in parafollicular or T-dependent compartments. Because nerve fibres do not enter germinal centres of follicles, the early stages of proliferation and differentiation of B-lymphocytes cannot be directly influenced (10, 13).

Nerve fibres, in the T-dependent areas are in close vicinity or in contact not only with migrating lymphocytes, plasma cells, magrophages and mast cells, but also with thin-wall arterial vessels, where they can participate in local modulation of humoral and cellular responses of the organism and indirectly in the overall immunological responses (2, 4, 3, 11).

The data, which we obtained on the focal non-neural AChE-positive area of the superficial lymphoepithelial layer of tonsils in rabbits, have not been found in the available literature. The authors suggest that this phenomenom may influence the processes of cellular migration across the superficial epithelial barrier of tonsils.

Our findings of nerve supply in the palatine tonsils in rabbits and cats are in agreement with those authors (2, 5, 4, 6), who have investigated innervation in other lymphoid organs in mammals, and found that AChE-positive nerve fibres innervate not only the vasculature but also the parenchyma of lymphoid organs with specific functional compartments and do not enter their B-dependent compartments.

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THE EFFECT OF PARACETAMOL ON MITOCHONDRIAL RESPIRATION AND ACTIVITIES OF SOME ANTIOXIDATIVE EZYMES IN RAT LIVER *IN VITRO*

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ABSTRACT

The effect of different concentrations of paracetamol on the mitochondrial respiration and activity of ATP-ase, glutathione reductase (GR) and glutathione peroxidase (GPx) was studied in rat liver mitochondria *in vitro*. Mitochondrial respiration and ATP-ase activity were inhibited only by a high paracetamol concentration (1 mg and 1.5 mg.mg^{-1} mitochondrial protein). The same paracetamol concentration increased the activity of GR and GPx.

Key words: ATP-ase; glutathione peroxidase; glutathione reductase; mitochondria; mitochondrial respiration; paracetamol

INTRODUCTION

Paracetamol (acetaminophenone) is one of the most frequently employed analgesics/antipyretics with a hepatotoxic effect. Paracetamol hepatotoxicity is probably caused by a product of its metabolism, N-acetyl-p-benzochinone-imide (NAPQI), that binds covalently to proteins (10). The administration of paracetamol also results in oxidative stress, the formation of peroxides (22) and a decrease in intracellular glutathione concentration (16). Multiple doses of paracetamol decrease its hepatotoxicity as a result of an adaptive and protective response of an organism to oxidative stress, indicated by an increase in glutathione reductase (GR) and glucose-6-phosphate dehydrogenase activity (20).

Previous studies with rat liver mitochondria have demonstrated that the *in vivo* effect of paracetamol on microsomal oxidation, mitochondrial respiration, phosphorylation and mitochondrial structure varies with the age and species of experimental animals (3) and is related to the dose of the drug (16, 23). Paracetamol *in vitro* in both, subtoxic and toxic doses causes morphological change and a dysfunction of mitochondria and subsequent changes in mitochondrial respiration and ATP production. In milimolar concentration paracetamol inhibits oxidative phosphorylation and mitochondrial respiration with a mechanism that is probably identical with mitochondrial respiratory chain uncoupling (18).

Oxidative stress is defined as a shift in equilibrium between the production and elimination of reactive oxygen species (ROS) towards an excessive ROS accumulation. Under normal physiological conditions ROS are eliminated by antioxidative enzymes as well as non-enzyme defense mechanisms.

Glutathione peroxidase (GPx) and glutathione reductase (GR) together with superoxide dismutase (SOD) are key enzymes of the antioxidative mechanism responsible for cell defense against oxidative stress.

It is known that the sensitivity of an organism to paracetamol is proportional to the dose of the drug and depends also on many other factors. This probably explains discrepancies in the published results on the drug's effect on antioxidative systems protecting cells against oxidative damage (2, 20).

In our study we have investigated the effect of different paracetamol concentrations on mitochondrial respiration, ATP-ase activity and GPx and GR activity *in vitro.*

MATERIAL AND METHODS

Female Wistar rats weighing 200—250 g fed on a standard laboratory diet and tap water were used in the experiment. The animals were killed by decapitation, their livers were quickly removed and the mitochondria were isolated according to Johnson and Lardy (13). Control and experimental groups consisted of the same number of samples. Paracetamol was administered in doses of 1 or 1.5 mg.mg^{-1} mitochondrial protein.

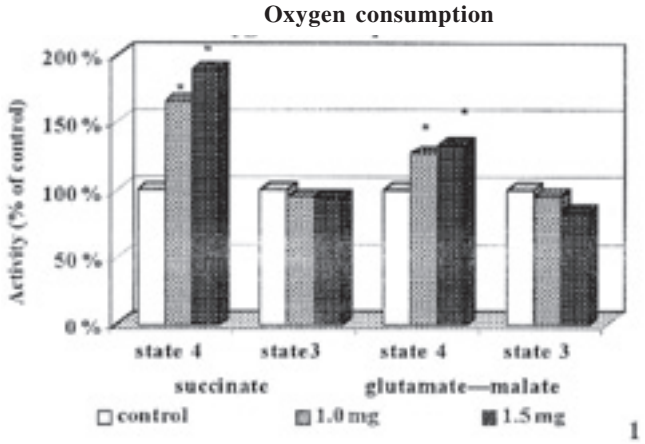

Fig. 1. Oxygen consumption in rat liver mitochondria (%) in dependence on concentration of paracetamol

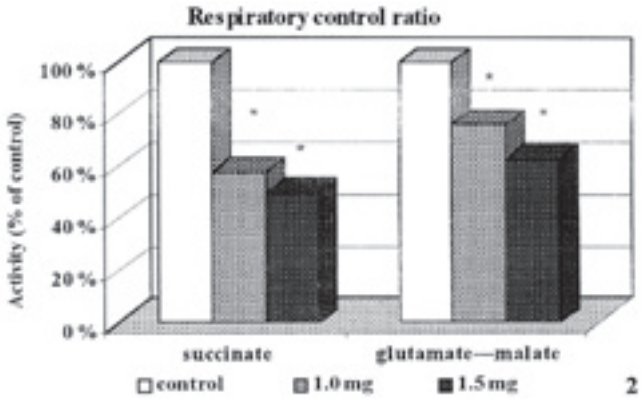

Fig. 2. Respiration control ratio of the rat liver mitochondria in the presence of glutamate and malate (%) in dependence on a concentration of paracetamol

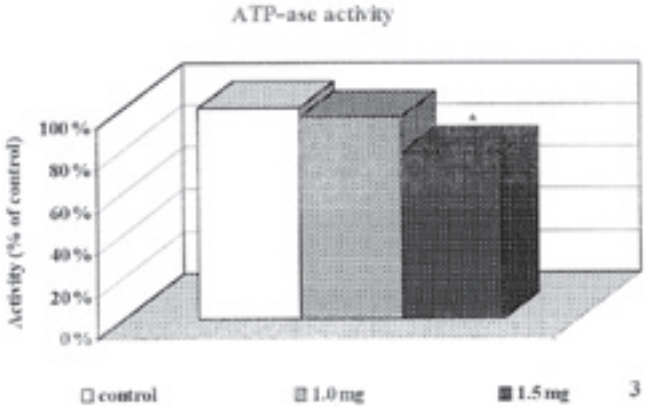

Fig. 3. Changes in ATP-ase activity in rat liver mitochondria (%) in dependence on a concentration of paracetamol

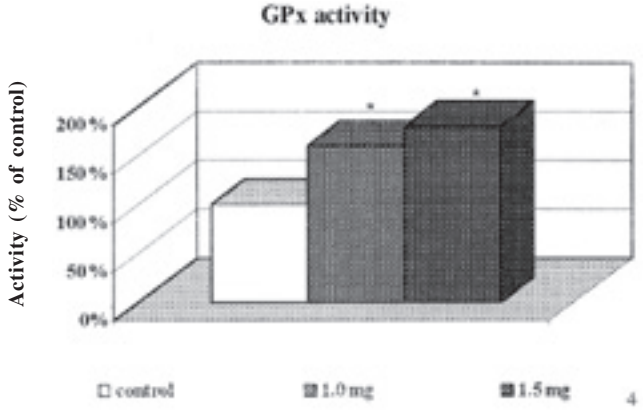

Fig. 4. Changes in glutathione peroxidase activity (GPx) in rat liver mitochondria (%) in dependence on a concentration of paracetamol

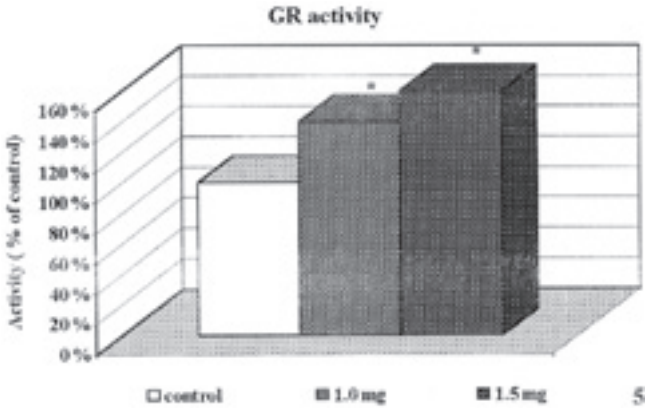

Fig. 5. Changes in glutathione reductase activity (GR) in rat liver mitochondria (%) in dependence on concentration of paracetamol

Mitochondrial respiration was determined by oxygen consumption measured by oxygen electrode (WTW, Germany) in state 4 without ADP and in state 3 after ADP addition. Respiratory control ratio (RCR) was determined as a ratio between oxygen consumption in state 3 and state 4.

ATP-ase activity (EC 3.6.1.3) was determined according to Meisner (17) and is expresssed in mmol Pi/mg protein/min.

GPx activity (EC 1.11.1.9) was measured by the consecutive glutathione reductase reaction. This reaction was monitored by the oxidation of NADPH assayed at 360 nm (9). The decrease in NADPH absorption at 360 nm was monitored for 5 min.

The activity of the enzyme was expressed in $U.kg^{-1}$ mitochondrial protein.

The activity of glutathione reductase (EC 1.6.4.2) was determined by the method of Calberg and Mannervick (5). The decrease in absorbance at 340 nm was monitored at 37 °C for 5 min. Glutathione reductase activity was expressed as the amount of enzyme which catalyzes the reduction of 1 mol of NADPH per second per kg of mitochondrial protein ($kat.kg^{-1}$).

Mitochondrial protein concentration was determined according to Hartree (11). The results were analyzed by Student's *t*-test with $p<0.05$ level considered as significant (designated by symbol *). Results are expressed as % of controls.

RESULTS

Tab. 1 and Fig. 1 show the increase in mitochondrial respiration in state 4 in the presence of a succinate and paracetamol application at 1 and 1.5 $mg.mg^{-1}$ mitochondrial

protein. The respiration in state 3 was not changed at both paracetamol concentrations. As a result RCR was significantly decreased by 42.4 % and 50.5 %, respectively at both paracetamol concentrations (Fig. 2).

An increase in state 4 respiration was observed also in the presence of glutamate and malate. No changes were observed in state 3 (Fig. 1). RCR decreased by 24.4 % at 1 mg concentration and by 37.2 % at 1.5 mg paracetamol concentration (Tab. 2, Fig. 2).

ATP-ase activity significantly decreased compared with the controls at 1.5 mg paracetamol mg^{-1} mitochondrial proteins (Tab. 3, Fig. 3).

GPx activity was influenced in a similar pattern as GR activity (Tab. 3, Fig. 4). In the presence of the drug in 1.0 a 1.5 $mg.mg^{-1}$ mitochondrial protein GPx activity increased by 60 and 80 %, respectively.

GR activity increased in the presence of paracetamol (Tab. 3, Fig. 5), with a significant difference in contrast with the controls (by 40, and 59 %, respectively) at 1.0 and 1.5 mg paracetamol.mg^{-1} mitochondrial protein.

Table 1. Respiration of rat liver mitochondria in dependence on a paracetamol concentration in the presence of succinate

succinate	control $\bar{x} \pm s_x$	1.0 mg $\bar{x} \pm s_x$	1.5 mg $\bar{x} \pm s_x$
O_2 consumption in state 4 ($mmol.min^{-1}.kg^{-1}$ prot.)	15.23 ± 0.24	25.17 ± 2.45	28.90 ± 0.68
O_2 consumption in state 3 ($mmol.min^{-1}.kg^{-1}$ prot.)	33.88 ± 0.65	32.32 ± 3.30	31.70 ± 2.70
RCR (state 3/state 4)	2.22 ± 0.20	1.28 ± 0.10	1.10 ± 0.09

Table 2. Respiration of rat liver mitochondria in dependence on a paracetamol concentration in the presence of glutamate – malate

glutamate – malate	control $\bar{x} \pm s_x$	1.0 mg $\bar{x} \pm s_x$	1.5 mg $\bar{x} \pm s_x$
O_2 consumption in state 4 ($mmol.min^{-1}.kg^{-1}$ prot.)	10.25 ± 1.05	13.05 ± 1.07	13.67 ± 1.20
O_2 consumption in state 3 ($mmol.min^{-1}.kg^{-1}$ prot.)	24.86 ± 1.50	23.92 ± 2.40	20.82 ± 2.55
RCR (state 3/state 4)	2.42 ± 0.10	1.83 ± 0.15	1.52 ± 0.20

Table 3. Activities of antioxidant enzymes in dependence on a paracetamol concentration

	control $\bar{x} \pm s_x$	1.0 mg $\bar{x} \pm s_x$	1.5 mg $\bar{x} \pm s_x$
Glutathione peroxidase ($U.mg^{-1}.kg^{-1}$ prot.)	0.35 ± 0.02	0.56 ± 0.05	0.63 ± 0.09
Glutathione reductase ($kat.kg^{-1}$ prot.)	43.81 ± 7.09	60.53 ± 9.64	68.88 ± 3.8
ATP-ase ($\mu mol.P_i.min^{-1}.mg^{-1}$ prot.)	0.38 ± 0.02	0.20 ± 0.02	0.30 ± 0.02

DISCUSSION

Paracetamol is metabolized in an organism by cytochrome P-450 to NABQI (8). This metabolite reacts spontaneously with glutathione yeilding conjugated compounds. NABQI is also known to form covalent complexes with oxidized glutathione and to reduce the cytochrome c level. Paracetamol itself can also react rapidly with oxidized glutathione forming conjugates (12, 19). Paracetamol *in vivo* in the toxic dose of (over 800 $mg.kg^{-1}$ weight) as soon as after 1 h decreases activity of glutamate dehydrogenase and this reaction is supposed to contribute to paracetamol toxicity (10). Knight *et al.* (14) have suggested that the reactive paracetamol metabolises by binding with intracellular proteins to produce superoxide radicals and ultimately mitochondrial dysfunction. As a result of the multiple paracetamole interactions there is a dose dependent depletion of glutathione (in the dose of 800 $mg.kg^{-1}$ to 20—25 % of a normal physiological level).

The effect of paracetamol in both, subtoxic and toxic doses on energy metabolism in liver mitochondria was studied mostly in experiments *in vivo*. The concentration of paracetomol (1.0 and 1.5 $mg.mg^{-1}$ mitochondrial protein) used in our *in vitro* experiments corresponds approximately to the subtoxic (375 $mg.kg^{-1}$ weight), and toxic doses (800 $mg.kg^{-1}$ weight) of the drug. Therefore the results may be compared with the paracetamol effect *in vivo* described in the published literature (6, 24).

In our *in vitro* experiments paracetamol affected mitochondrial respiration by a significant decrease in RCR. The toxic effects of paracetamol on the respiration of hepatic mitochondria in isolated mouse hepatocytes has also been observed by Burcham and Harman (4). They have found that the loss of mitochondrial respiratory function was accompanied by a decrease in ATP. Respiratory complex II containing succinate dehydrogenase showed a high sensitivity to paracetamol. Mitochondrial dysfunction determined by a decrease in RCR after paracetamol *in vivo* was observed also by Donelly *et al.* (6).

SOD is an antioxidative enzyme acting in the first line of cell defence against oxidative damage (15). H_2O_2, a product of superoxide detoxification by SOD is a substrate for GPx in a reaction requiring reduced glutathione and producing oxidized glutathione. Accumulation of oxidized glutathione is prevented by the action of GR in an reverse reaction yielding a reduced form of glutathione. Increase in GPx activity after 1.0 and 1.5 mg paracetamol. mg^{-1} mitochondrial protein in our study is in accordance

with the increase of GPx activity in hepatocytes after paracetamol administration *in vivo (8).*

The increase in GPx activity is considered a result of ROS production that is in vitro accompanied by mitochondrial damage and by the collapse of mitochondrial trans-membrane potential. Paracetamol *in vitro* at a dose higher then the therapeutic also caused the activation of erythrocyte antioxidant enzyme including GPx (21). In contrast, Binda *et al.* (3) observed a decrease in GPx activity in isolated hepatocytes in the presence of paracetamol.

In addition to GPx, the activity of mitochondrial GR also increased in the presence of paracetamol (1.0 and 1.5 mg of the drug.mg^{-1} mit proteins). This is in accordance with results published by Adamson *et al.* (1), who reported an increase in GR activity and a concomittant decrease in reduced glutathione (GSH) and an increase in oxidized glutathione (GSSG) concentration in isolated hepatocytes after paracetamol administration *in vitro.* Similarly, GR activity increased by 61 and 62 % respectively and glucose-6-phosphate dehydrogenase by 130/110 %, after paracetamol administration at a dose of 800 and 1200 mg.kg^{-1} *in vivo (20).*

Paracetamol belongs to the group of compounds with hepatotoxic effects that trigger radical reactions proportionately to the dose and duration of the administration of the drug. This unfavorable effect can be accelerated by the depletion of antioxidants. The effect of oxidants in general, can be depressed by antioxidative therapy. As for as paracetamol, a good antioxidant is vitamin E, which increases GR activity and returns the reduced glutathione level to almost normal levels (26). Some derivatives of vitamin E also have very similar properties that exhibit antioxidative properties and a membranotrophic effect, decrease serum aminotransferases and superoxide radical levels and increase the activity of catalase and reduced glutathione levels (25). The selection of antioxidant needs to be very careful, also taking into consideration its possible effect on other metabolic systems (7).

Our experiments fit well into the definition of paracetamol as an ROS producing compound and further confirm previous observation that paracetamol administration results in liver damage and in the changes in antioxidative enzymes only in the drug's dosage that are therapeutically considered to be toxic.

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THE INFLUENCE OF CONTINUOUS GAMMA RADIATION ON HISTONES IN THE LIVER OF RATS OF DIFFERENT AGES

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ABSTRACT

Age-related changes in histones in the intact and regenerating liver of rats continuously irradiated (^{60}Co) with a total dose of 12.6 Gy (daily dose rate of 0.6 Gy) of gamma rays were examined. Quantitative histone changes in rats (from 1 to 9 months of age) were found to be mild only. In the total extracted histones there was a decrease in proportion of the H1 histone fraction with preceding increase in proportion of the H1^0 subfraction. Thirty minutes after irradiation the amount of histones was reduced in the intact liver depending on age, probably due to the impaired extractibility of histones. Similar patterns of changes in proportion of the H1 fraction and H1^0 subfraction were observed in irradiated and non-irradiated animals, in the former with earlier onset. Irradiation, therefore, accelerated spontaneous age-related alterations, of rat liver histones.

Key words: aging; histones; irradiation; rat liver; regeneration

INTRODUCTION

The aging process is a physiological phenomenon which affects all individuals of all organisms. The process is accentuated and accelerated by some injurious agents and finally terminated due to the increased susceptibility and decreased resistance associated with ageing processes.

Histones play an essential role in the structure and function of the chromatin of all eukaryotic cells (20). The pattern of chromatin nucleosomal core histones (H2A, H2B, H3 and H4) seems to be relatively stable during the development and ageing of organisms. However, in the slowly proliferating liver tissue of ageing mice, the change in proportion of basal and replication variants of the histones H3 and H2A was found (12). In the less conserved H1 histone fraction a relative proportion of the H1^0 variant (which occurs in the chromatin of non-proliferating and terminally differentiated cells, e.g. hepatocytes in rodents) increases with age (12,13,19). H1^0 is one of the histone variants of the H1 family. Like H1, H1^0 is located on the linker region of nucleosome and is associated with an increased resistance of chromatin, which suggested that H1^0 could be associated with, or responsible for a more compact conformation of chromatin.

In previous papers we have studied changes in histones and DNA induced by the ageing and single whole body irradiation (7, 8, 9).

With regard to some common features between age-related and radiation induced alterations we have tried to find if any accumulation of apparent or latent changes occurs in the slowly proliferating liver cells. Therefore, we have investigated the changes of histones in the intact and regenerating liver of rats continuously irradiated with a total dose of 12.6 Gy (daily dose rate of 0.6 Gy for 21 days) and non-irradiated rats aged one, three and nine months.

MATERIAL AND METHOD

In this experiment male Wistar rats aged one, three and nine months were used. The animals were quarantined for a period of two weeks and were housed in cages at temperature of approximately 23 °C. They were fed *ad libitum* with a commercial laboratory rat diet (LD pellets, Velaz, Prague) and watered with tap water. The animals were divided into six groups, each of five animals. Research was conducted according to the principles enunciated in the *"Guide for the Principles Use of Laboratory Animals"* prepared by State Veterinary Office of The Slovak Republic, Bratislava. To eliminate circa-annual and

circadian variation effects experiments were always performed in autumn, at the same time between 7.30—9.00 a.m.

Groups of five rats of different age were continuously irradiated by gamma rays (^{60}Co) on the experimental gamma field (17) at the daily dose rate of 0.6 Gy for twenty-one days. The total dose was 12.6 Gy. The groups of control animals of the same ages as the irradiated rats were at the same time settled within the experimental gamma field, but they were kept beyond the reach of the ionizing radiation.

Irradiated animals were subjected to partial (70 %) hepatectomy, together with non-irradiated control animals. The operation was performed thirty minutes after the end of irradiation under light ether anaesthesia with a standard procedure. The rats were investigated at the 30th post-operative hour, at the time when the first synchronized wave of DNA synthesis was completed in the regenerating liver remnant (1, 5).

The isolation of nuclei and histone extraction were done according to the method of Grunicke *et al.* (3).

Protein concentration was determined by the method of Lowry *et al.* (11) using bovine serum albumin as a standard.

Electrophoresis was carried out using the method of Panyim and Chalkley (16). Histones were stained with amido black B. The relative proportion of individual histone fractions was determined spectrophotometrically on a densitometer, Shimadzu CS-930 (Japan).

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

RESULTS

Liver weight. Age related alterations of intact liver included a significant increase in the total organ wet weight in non-irradiated rats. The increase was slower in irradiated rats (Fig. 1A). After partial hepatectomy, the enlargement of liver remnant proceeded quickly in young, non-irradiated animals, especially in the one month-old rats. In these animals, the weight of the regenerating liver remnant reached 78 % of pre-operative values (*vs* 30 % left at operation). Despite this, the weight of the regenerating liver of all the rats (irradiated and non-irradiated of different ages) thirty hours after partial hepatectomy was significantly lower comparing to the weight of an intact liver. In continuously irradiated young rats the enlargement of the regenerating liver was markedly inhibited (Fig.1B).

Histones. Changes of the histone concentration per gram wet weight were not statistically significant. Nevertheless a tendency to decrease due to irradiation was evident in the intact liver (Figs. 2A, 2B).

The total histone content in the intact liver of non-irradiated rats quickly increased in the course of the first three months of life. In the regenerating liver the most rapid increment of the total histone content was observed in rats at the age of one month. Irradiation significantly decelerated the increase in the total histone content in both intact and regenerating liver especially in younger

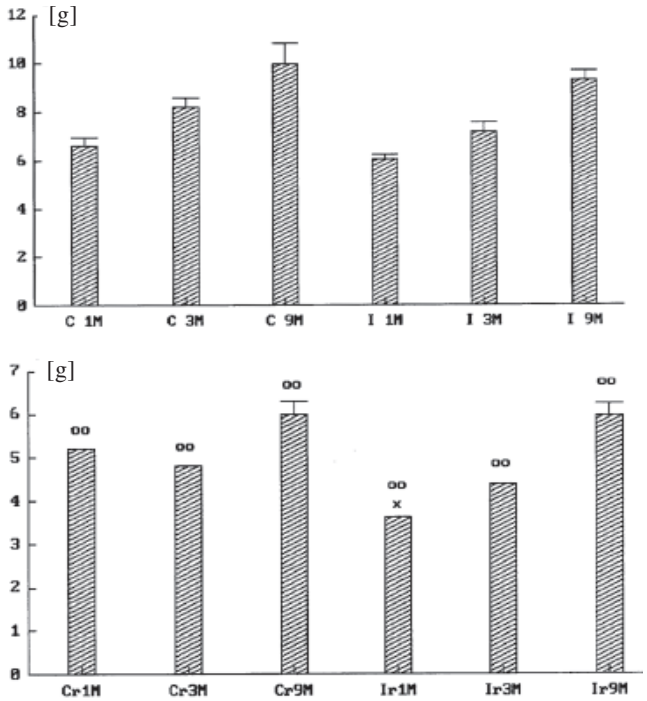

Fig. 1. Weight of the intact (1A) and regenerating (1B) liver of non-irradiated (C) and gamma-irradiated (I) (12.6 Gy — daily dose rate of 0.6 Gy) rats aged one, three or nine months

C — 1M, 3M, 9M: control one, three or nine month old rats, respectively

C_r — 1M, 3M, 9M: control one, three or nine month old rats, respectively, thirty hours after partial hepatectomy

I — 1M, 3M, 9M: continuously irradiated rats aged one, three or nine month old rats, respectively

I_r — 1M, 3M, 9M: continuously irradiated rats aged one, three or nine month old rats, respectively, thirty hours after partial hepatectomy

x — $P \leq 0.05$; xx — $P \leq 0.01$: for differences between irradiated and control rats

o — $P \leq 0.05$; oo — $P \leq 0.01$: for differences between regenerating and normal livers

animals (Figs. 3A, 3B). In contrast, the total histone content in the regenerating liver (thirty hours after partial hepatectomy) was lower compared to that in the intact liver of rats of all groups.

The relative proportion of the individual histone fractions was shifted for some deficit of the histone H1 and H4 to the benefit of the histones H2 + H3 (Table 1). No unambiguous changes in the proportion of histone fractions accompanied the ageing of rats or irradiation. The histone variant $H1^0$ within the fraction H1 in the intact liver increased from 8.8 % in one month old rats to 13 % in three and nine month old rats. After irradiation the increase in $H1^0$ variant in intact liver was more rapid and continued longer than in non-irradiated rats of comparable age (from 6 % to 32 %) (Table 2). In the regenerating liver, the alterations of $H1^0$ histone variant in the course of ageing and after irradiation were smaler than in the intact liver.

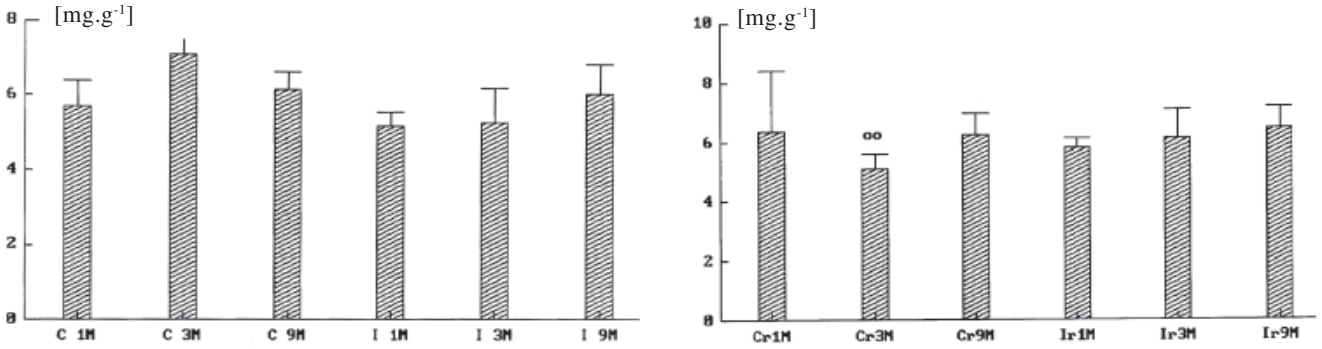

Fig. 2. Concentration of histones in the intact (2A) and regenerating (2B) liver of non-irradiated (C) and gamma-irradiated (I) (12.6 Gy — daily dose rate of 0.6 Gy) rats aged one, three or nine months (For explanation see Fig. 1)

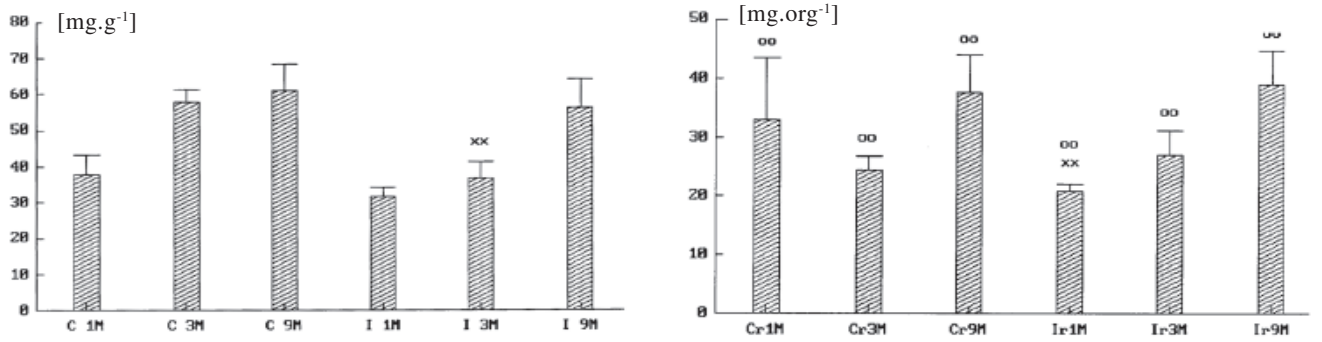

Fig. 3. Total content of histones in the intact (3A) and regenerating (3B) liver of non-irradiated (C) and gamma irradiated (I) (12.6 Gy — daily dose rate of 0.6 Gy) rats aged one, three and nine months (For explanation see Fig. 1)

Table 1. Relative proportion of histone fractions (H1, H2 + H3, and H4) in the intact and regenerating liver of non-irradiated and gamma-irradiated rats aged one, three or nine months

Age (months)	Intact			Regenerating		
	H1	H2+H3	H4	H1	H2+H3	H4
Control						
1	**33.19 ± 3.96**	**55.97 ± 0.63**	**11.75 ± 3.52**	**33.19 ± 2.90**	**57.02 ± 1.01**	**11.89 ± 1.15**
3	**29.70 ± 2.48**	**63.49 ± 0.92**	**5.56 ± 4.03**	**29.70 ± 1.59**	**59.97 ± 3.62**	**15.59 ± 2.36**
9	**29.30 ± 2.36**	**59.74 ± 1.24**	**10.95 ± 1.81**	**29.30 ± 1.83**	**59.85 ± 6.56**	**7.91 ± 3.60**
Irradiated						
1	**31.12 ± 0.11**	**62.52 ± 3.52**	**4.29 ± 0.76**	**30.17 ± 3.57**	**59.85 ± 3.57**	**10.20 ± 2.83**
3	**29.63 ± 2.63**	**61.95 ± 4.50**	**8.35 ± 2.90**	**26.14 ± 1.10**	**61.70 ± 8.34**	**3.89 ± 1.77**
9	**32.28 ± 6.50**	**61.82 ± 3.62**	**6.21 ± 2.20**	**23.69 ± 0.74**	**68.70 ± 0.84**	**7.72 ± 0.33**

Table 2. The percentage of $H1^0$ variant within the H1 histone fraction of the intact and regenerating liver of non-irradiated and gamma-irradiated rats aged one, three and nine months

Age (months)	Intact Control rats $H1^0$	Regenerating Irradiated rats $H1^0$
Normal liver		
(1)	8.8 ± 0.6	5.9 ^{xx} ± 0.2
(3)	12.9 ± 3.8	12.8 ± 2.2
(9)	12.6 ± 1.7	31.8 ^{xx} ± 8.6
Regenerating liver		
(1)	**10.7 ± 1.4**	**11.9 ± 0.3**
(3)	**21.2 ± 6.7**	**11.7 ± 3.2**
(9)	**12.5 ± 1.7**	**19.3 ± 2.1**

DISCUSSION

In previous papers we have studied the effect of age and single whole body irradiation on the intact and regenerating rat liver on the basis of quantitative changes in histones and DNA (7, 8) and histone acetylation (9). We have found that during the course of regeneration induced by partial hepatectomy, age-related changes in the liver manifested themselves in decreasing the cellularity, slowing down the increase in DNA and histone content, changing the mutual proportions of histone fractions H1, H2A+H2B, H4 and increasing the $H1^0$ variant within histone H1.

Within nuclei isolated from intact and regenerating livers of rats, acetyl-transferase activity of histones increased with age. An age-dependent decrease in histone acetyltransferase activity in the normal and regenerating rat livers was obvious at the time of maximum activity (i.e. at the 4th min of incubation). The radiation-induced drop in acetyltransferase activity was mild in the intact liver. Twenty-four hours after partial hepatectomy, acetyltransferase activity in the regenerating liver was almost completly inhibited by irradiation.

Radiation-induced latent injury (a single dose of 5.7 Gy gamma radiation 30 min before partial hepatectomy) was manifested during induced regeneration in similar but much more profound changes with their earlier onset than those accompanying ageing. Since the changes in the regenerating liver were found not to be milder than in the normal liver it mean, that induced proliferation did not lead to elimination of altered cells or to their rejuvenation.

During continuous irradiation even at a low dose rates, the accumulation of damage took place in slowly proliferating hepatocytes. The liver tissue therefore, is incapable of forming a temporary stabilized state in the course of continuous irradiation (6,10,14) and shows a lower ability to adapt to radiation than most other tissues. The reason lies probably in the fact that contrary to the tissues proliferatively active, such as the bone marrow, spleen or testes, the liver cannot fully eliminate damaged cells by mitotic division.

Our results showed that radiation-induced changes of histones in the rat liver were similar to those which arise in the course of ageing; these changes were cumulative and led to the inhibition of age-dependent increase of liver weight and histone content in the intact liver. In the liver regenerating after partial hepatectomy, radiation changes were more profound, especially in the youngest animals as in the intact liver. In general, results obtained after continuous gamma irradiation (dose rate 0.6 Gy/day, total accumulate dose 12.6 Gy) were similar to those after single whole-body gamma irradiation (5.7 Gy).

The proportion of $H1^0$ variant within the class of H1 histone fraction had begun to increase already in the youngest animals. The $H1^0$ histone variant plays an important role in gene repression (13, 21). In quiescent tissues its proportion increases in inverse correlation with the mitotic activity in the course of ageing (2, 15, 18). However, age dependent accumulation of $H1^0$ histone variant, in the regenerating liver was similar or even higher than in the intact liver in spite of a multiple increase in the mitotic activity.

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THE INFLUENCE OF SELENIUM, pH AND INTRAMUSCULAR FAT CONTENT ON DRIP LOSSES IN BEEF

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ABSTRACT

The objectives of this study were to evaluate the effect of selenium on beef drip losses and fluid losses from vacuum packaged beef in relation to their pH value and intramuscular fat level and to evaluate the increase of selenium concentration in muscle in relation to the duration of supplementation. A total of 17 Holstein bulls were slaughtered. Group I (7 bulls) was used as a control group. Peroral selenium was given to the bulls in groups II and III (each 5 bulls) 7 and 30 days before slaughter. A sample of *m. longissimus dorsi* was taken from each carcass 24 hours after slaughter. Fluid losses from vacuum packaged beef were determined after 4 and 8 days storage. All the samples were analysed for their pH value, dry matter, protein, intramuscular fat and selenium. The selenium concentration had the greatest influence on losses (P = 0.085). The selenium concentration in relation to a higher pH was attributed to the decrease in fluid losses (0.17—0.20 %) of meat stored for 4 days. The fluid losses of meat stored for 8 days were lower when the values of pH, selenium, or intramuscular fat content were higher. The lowest losses came from meat with a higher selenium or intramuscular fat concentration (0.34 %). The selenium level increased with the supplementation time. Selenium concentrations in the muscles (in wet tissue) of bulls in each group were 0.107 ± 0.083; 0.168 ± 0.215 and 0.223 ± 0.175 mg.kg^{-1}.

Key words: antioxidants; Holstein bulls; vacuum packaging; water-holding capacity

INTRODUCTION

The ability of meat to retain inherent water is an essential meat quality parameter for economic, technological and sensory reasons. The water-holding capacity (WHC) is an important quality characteristic, which is often assessed by determining drip loss. Common drip losses from meat are 0.1—1 % after two days of storage.

The amount lost from sliced meat is higher (2—10 %). Very high losses come from thawed meat (about 25 %). The drip is formed by intracellular and extracellular water (in the ratio of 10 : 1), and contains proteins (80—160 mg.ml^{-1}), ions (124 mmol K^+, 21 mmol Na^+, 3 mmol Cl^-) and compounds of low molecular weight (amino acids, vitamins). The red colour of drip fluid is caused by the myoglobin content (9).

The amount of fluid loss is significant for the vacuum packaging of meat. Drip losses are influenced by many factors, for example the size of meat (higher losses come from sliced meat and steaks than from whole muscle), time *post mortem*, the storage temperature, etc. An important factor influencing drip loss is the pH value of meat. The rate of *post mortem* pH fall is a determinant of water-holding capacity.

A fast rate of pH fall will increase the tendency of the actomyosin to contract as it forms and thus express to the exterior fluid, which has become dissociated from the proteins (7). Highly significant correlations were found for the ultimate pH with drip loss (3, 11). Muscles having a high content of intramuscular fat tend to have a high water-holding capacity. The reasons for this effect are unknown. Possibly the intramuscular fat loosens up the microstructure, thus allowing more water to be retained (7).

Selenium is an important trace element. Its most significant function in the organism is its antioxidant influence. The beneficial effect of antioxidants on meat quality is related to lower lipid oxidation, enhanced colour stability and lower drip losses during storage, all of which enhance the possible storage time of the products. Selenium is a part of glutathione peroxidase. This enzyme, in relation with vitamin E, protects biological membranes from the harmful effect of free radicals. The protection of cell membranes from oxidation is the reason for the retention of water in meat and the decrease in drip losses (13).

Selenium plays an important role in the health of animals and people. A low concentration of selenium in animal tissues has been linked to some diseases (white muscle disease, exsudative diathesis, etc.). A sufficient supply of selenium to animals is important for its health and for optimal level of this element in animal products, it becomes a natural source of selenium for humans.

The objectives of this study were to evaluate the effect of selenium on beef drip losses and fluid losses from vacuum packaged beef in relation to their pH value and intramuscular fat level and to evaluate the increase of selenium concentration in muscle in relation to the duration of supplementation.

MATERIALS AND METHODS

A total of 17 Holstein bulls were chosen for the experiment. The average age of the bulls was 22 months. The bulls were housed and tethered. The diet consisted of the typical feed of the region (maize silage, grain meal, extracted meal, minerals and vitamins). The feed ration was calculated according to recommendations of Sommer *et al.* (12) for a daily gain of 1000 g.

The animals were divided into three groups. Group I (7 animals) was the control group. Selenium was given to the bulls in groups II (5 animals) and III (5 animals). Peroral selenium was given using SEL-PLEX 50 (ALLTECH) (selenium in its organic form as a Se-methionine, Se-cysteine and other Se-amino acids) in 4 mg doses per day. Group II received these dosages for 7 days, and group III for 30 days, before slaughter. All the bulls were slaughtered at a commercial abattoir.

A sample of *m. longissimus dorsi* was taken from each carcass 24 hours after slaughter. The pH value was measured in each sample using a SENTRON ARGUS pH-meter with an ISFET probe. The muscle was divided into three parts (approx. weight 2.5 kg). The first part was used for drip loss determination and other chemical analyses. The second and third parts were used for fluid loss determination and so were vacuum packaged. PA-PE bags were used for packaging. The difference between the weight of the fresh samples and weight of the vacuum packaged beef, after 4 and 8 days of storage at 4 °C (VP-4 and VP-8), determined the fluid losses.

Meat samples for determination of drip loss about 150 g were put into PE bag. These samples were stored at 4 °C for 24 hours. Drip loss was expressed as a percentage of the initial weight of the meat constituted to exsudate. All samples were analysed for dry matter, protein and intramuscular fat content. The dry matter content was determined by drying samples at 105 °C to a constant weight. Protein and intramuscular fat content was assessed using Kjeltec and Soxtec apparatus (TECATOR), respectively. The selenium content was determined by taking 0.5 g of a wet sample. After adding of 5 ml nitric acid and 2 ml hydrogen peroxide the sample was mineralized in a microwave system MLS 1200 Standard. The selenium was than determined using the AAS method with electrothermical atomization in the Solaar M6 (THERMO ELEMENTAL). The results are expressed in $mg.kg^{-1}$ of wet tissue.

All the data was analysed using the UNISTAT 4.53 program. The average values of the observed parameters and their variability were calculated using common procedures. A three-factorial analysis of variance was applied to assess the above-mentioned effects of pH, intramuscular fat and selenium content.

Table 1. Carcass weight, chemical composition of muscles, drip losses and selenium concentration in muscles within each group

Trait	I		II		III	
	Mean	SD ±	Mean	SD ±	Mean	SD ±
Carcass weight, kg	370.00	14.34	377.20	33.44	398.00	18.27
Drip loss, %	0.98	0.53	0.17	0.31	0.50	0.23
VP-4, %	0.25	0.17	0.45	0.19	0.03	0.04
VP-8, %	0.76	0.42	0.40	0.21	0.65	0.39
Dry matter, $mg.100\,g^{-1}$	26.68	1.74	29.28	1.70	26.50	0.91
Proteins, $mg.100\,g^{-1}$	20.87	0.85	20.44	1.02	21.23	0.70
IMF, $mg.100\,g^{-1}$, [a]	5.14	1.83	8.25	2.29	4.72	1.31
pH	5.71	0.35	5.56	0.22	5.94	0.50
Selenium, $mg.kg^{-1}$	0.107	0.083	0.168	0.215	0.223	0.175

[a] — Intramuscular fat

Table 2. The influence of selenium, pH and intramuscular fat on drip losses and the chemical composition of muscle

Trait	Mean	SD (±)	Se		pH		IMF[a]	
			< 0.12	> 0.12	< 5.6	> 5.6	< 5	> 5
Number of carcasses			8	9	10	7	7	10
Carcass weight, kg	379.30	26.57	375.60	382.60	318.80	375.70	385.70	374.80
Drip loss, %	0.87	0.50	0.94	0.82	0.90	0.82	0.89	0.85
VP-4, %[b]	0.24	0.23	0.26	0.25	0.30	0.20	0.22	0.29
VP-8, %[b]	0.59	0.39	0.78	0.42	0.66	0.50	0.63	0.57
Dry matter, mg .100 g^{-1}	27.20	1.93	27.40	27.00	27.00	27.40	25.70	27.40
Proteins, mg . 100 g^{-1}	20.80	0.92	20.70	20.90	20.90	20.60	21.30	20.10
IMF, mg . 100 g^{-1}, [a]	5.80	2.49	6.30	5.30	5.80	5.90	3.60	7.40
pH	5.75	0.41	5.55	5.90	5.50	6.10	5.90	5.70
Selenium, mg. kg^{-1}	0.16	0.17	0.04	0.27	0.15	0.17	0.09	0.22

[a] — Intramuscular fat; [b] — Fluid loss from vacuum packaged meat stored for 4 or 8 days

RESULTS AND DISCUSSION

Carcass weight, chemical composition of muscles, drip losses and selenium concentration in muscles within each group are shown in Table 1. The selenium level increased with the duration of supplementation. Concentrations in all samples ranged from 0.0002 to 0.583 mg.kg^{-1}. The average concentration in the whole group was 0.159 mg.kg^{-1} (SD = 0.172). The selenium level in bulls' muscles without supplementation (group I) was not high.

In some countries, the concentrations in beef are between 0.01 (Finland – before selenium application to soils) and 0.27 mg.kg^{-1} (USA). In The Czech Republic the published concentration value is 0.02 mg.kg^{-1} (14). Ingr *et al*. (6) found selenium concentrations in the muscles of bulls from south Moravian region to be between 0.06—0.40 mg.kg^{-1}. Pavlata *et al*. (10) reported that the selenium concentration in the muscles of calves was 0.092 mg.kg^{-1}. The influence of supplementation was found, but the increase in the selenium level in muscles was not high. The reason for this low concentration of selenium after treatment is probably the result of a deficiency of selenium in the feed, because after supplementation the other tissues (kidney, liver) were saturated.

For statistical analyses all carcasses were divided into two groups according to their pH values, selenium concentration, or intramuscular fat content. The averages of the following parameters within each group were counted. The average values of drip losses, fluid losses from vacuum packaged meat, pH values and the chemical composition of muscles are given in Table 2.

In comparison with other published values of drip losses, these assessed drip losses were not high. French *et al*. (5) described drip losses from beef as being between 2.11—2.73 %. Drip losses from Holstein bulls ranged from 0.59 to 1.57 % (1). Drip losses were the most influenced by a selenium concentration; while a lower value was found in muscles with a higher selenium concentration, this influence was not significant. The highest drip losses occurred in muscles with low intramuscular fat and selenium content (1.48 %) and with low intramuscular fat and pH value (1.14 %). The lowest drip losses were 0.57 %.

There are no big differences in fluid losses from vacuum packaged meat that was stored over four days. The lowest fluid losses were in samples with a higher pH value. The influence of selenium concentration was found (but it was not significant). Selenium concentration with a higher pH had a positive effect on the amount of fluid losses (0.17—0.20 %). The effect of intramuscular fat was with a higher pH (0.20 %).

As expected, fluid losses from vacuum packaged beef stored over eight days were higher than from meat stored for four days. The positive effect of all three following parameters was found. The fluid losses were lower when the values of pH, selenium, and intramuscular fat content were higher. The lowest losses were from meat with higher intramuscular fat and selenium concentrations (0.34 %). The greatest influence on losses was the selenium concentration (P = 0.085).

Similar differences in drip losses from pork in control and selenium supplemented groups of animals were published by Torrent (13). Drip losses increased with the period of storage. Higher differences were found in steaks than in whole muscles. Data from the literature describing the values of drip losses from beef (or fluid losses from vacuum packaged meat) after selenium supplementation were not available.

Table 3. Correlation coefficients for drip losses

	pH	Se	IMF[a]	Supl.[b]
Drip loss	-0.42	-0.16	-0.01	-0.44
VP-4[c]	-0.47	-0.01	0.04	-0.58*
VP-8[c]	-0.29	-0.29	-0.17	0.01

* — $P < 0.05$
[a] — Intramuscular fat
[b] — Supplementation
[c] — Fluid loss from vacuum packaged meat stored for 4 or 8 days

Correlation coefficients for losses and the following parameters and duration of supplementation are shown in Table 3. Negative correlations were found among losses and pH value. A significant ($P<0.05$) negative correlation was found between duration of supplementation and fluid losses from vacuum packaged meat stored for four days. A correlation coefficient of 0.61 ($P<0.05$) was found between drip loss and fluid loss from vacuum packaged meat stored for four days.

The influence of selenium on drip losses was shown. The effect of selenium was higher in connection with a higher pH value and intramuscular fat content. Differences were not significant, because of the lower number of animals. To increase the water retention in meat it is possible to give animals selenium in combination with other antioxidants, for example with vitamin E, which is often used for the improvement of beef quality (especially for colour stability) (4, 8). Another possibility for decreasing drip loss from meat is to supplement some elements. The drip loss from pork was lower from animals that were supplemented with magnesium (2). The effect of magnesium or other elements on drip losses from beef has not been investigated yet.

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HEPATIC CAPILLARIASIS IN THE MEDITERRANEAN BARBEL *(Barbus meridionalis Petenyi* Heck.) FROM LAKE OHRID

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SUMMARY

Hepatic capillariasis in fish is known to cause serious liver damage and has a possible impact on health which may be of veterinary concern in economically important feral or cultivated species. In this study, the liver histopathology of the Mediterranean barbel infected by the capillariid nematode *S. petruschewskii* was described. Particular attention was paid to the cholangiopathic changes observed for the first time in association with fish hepatic capillariasis.

Key words: cholangiopathy; hepatic capillariasis; histopathology; Mediterranean barbel *(Barbus meridionalis petenyi)*

INTRODUCTION

Hepatic capillariasis in freshwater fish is caused by a few nematode species whose adults reside in the host liver. Histopathological studies have revealed variable liver damage characterised by a broad range of lesions in hepatic and associated pancreatic tissues, depending on the degree and age of infection and fish species (2, 4, 8). Considering that hepatic capillariasis may impact on fish health, and therefore is a disease with veterinary and economic significance, an understanding of host-parasite interactions and associated pathology is important. In this study, the unusual liver pathology of Mediterranean barbel (*Barbus meridionalis petenyi* Heck.) from Lake Ohrid infected by a capillariid nematode is documented.

Short running title: Hepatic capillariasis in Mediterranean barbel

MATERIALS AND METHODS

A total of 147 adult Mediterranean barbels (90 males and 57 females, 112—283 mm fork length) were collected in overnight nets from several sites of Lake Ohrid (Balkan Peninsula). The fish were killed by cranial stunning followed by the severing the spinal cord and exsanguination. Liver samples were isolated, fixed in 10 % buffered formalin and processed by the standard paraffin method for routine histopathology. In addition, 1—2 mm tissue cubes were retrieved from selected paraffin blocks, reprocessed, resin embedded, and semi-thin sections prepared for high resolution light microscopy (11).

RESULTS AND DISCUSSION

The histopathological examination of liver specimens revealed capilariid infection in some of the collected barbel individuals. Embryonated nematode eggs with two polar plugs and adult worms were observed in liver tissue sections (Figs. 1a, b) According to the classification of the parasitic nematodes of freshwater fish of Europe (6), *Schulmanela petruschewskii* was assumed as the most likely nematode infecting the barbel in the present study. However, specific identification remains to be ascertained.

Capillariid eggs or adults were in evidence histologically in 37—66 % of the sampled individuals depending on the collection site. However, the prevalence is likely to be underestimated because only one to three tissue blocks per animal (1 section per block) were examined.

For the purpose of this study, only findings relevant to the presence of capillariid infection and associated lesions are reported. The characteristic barrel-shaped eggs, with two polar plugs and fully developed embryo

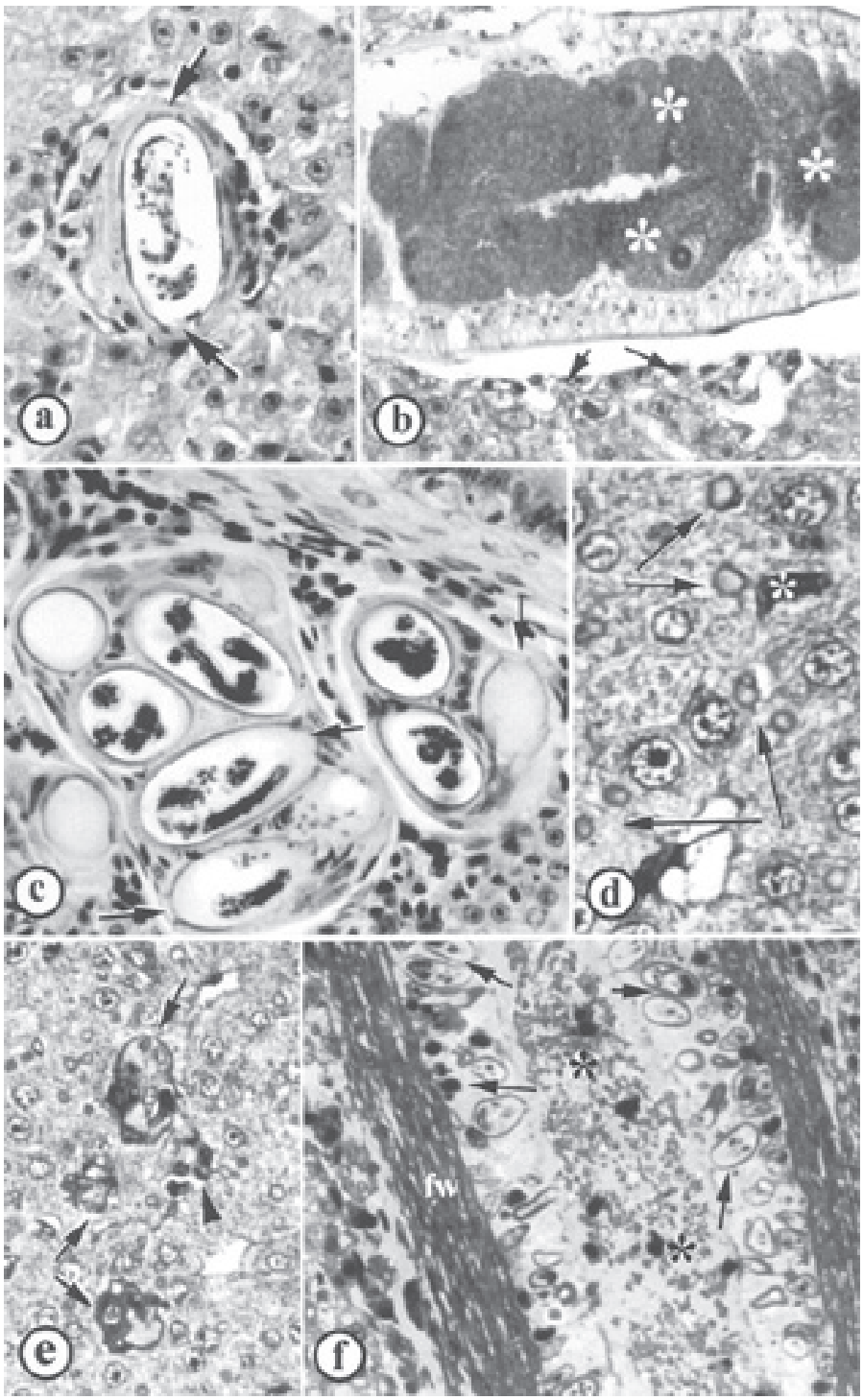

Figure 1. Liver lesions in Mediterranean barbel harbouring capillariid infection

a. **An encapsulated barrel-shaped egg with two polar plugs (*arrows*) containing a fully developed embryo; *b.* An adult worm in liver tissue tangentially sectioned through the stichosome. Note three rows of stichocytes (*asterisks*). Mild inflammatory response in the surrounding parenchyma (*arrows*); *c.* A cluster of capillariid eggs deposited in the vicinity of a biliary duct. Some polar plugs are visible (*arrows*); *d.* Intrahepatic cholestasis with dense bile plugs (*asterisk*) and dilated canalicules between and within hepatocytes (*arrows*); *e.* A group of biliary ductules with degenerating epithelium (*arrows*), two are completely collapsed (lower left corner). Intralobular infiltration of leukocytes (*arrowhead*); *f.* Longitudinal section through a large bile duct with "onion-skin" appearance of the fibrous walls *(fw).* Biliary epithelium heavily infiltrated with inflammatory and rodlet cells (*arrows*). Note the necrotic detritus in the lumen (*asterisks*). Paraffin sections, H&E *(a—c)*; semithin sections, toluidine blue *(d—f)*; × 400 (*a, b, c, e, f)* and × 1000 *(d)***

(Fig. 1a), grouped usually in clusters (Fig. 1c), deposited near blood vessels or bile ducts were found. This finding was occasionally accompanied by the observation of adult worms in the liver tissue of infected animals (Fig. 1b).

Two kinds of multifocal lesions were associated with the infection: severe necrosis along the migratory routes of adults, and strong granulomatous inflammations around capillariid ova. The acute inflammatory response provoked by freshly deposited eggs was observed as having developed into large granulomas consisting of concentric layers of epitheloid macrophages and eosinophils. In older capillariid infections, multiple fibrogranulomas in the final inflammatory stage, encapsulating degenerated or intact emryonated eggs, were observed. These lesions are in agreement with the histopathological findings reported for various freshwater fish suffering from hepatic capillariasis (2, 4, 8).

In this study, an additional lesion category affecting the biliary tree was consistently detected in infected barbel livers. Necrosis of biliary epithelium (Fig. 1e), periductal and intraductal inflammatory cell infiltration (Fig. 1f), proliferation of small biliary ducts, canalicular cholestasis (Fig. 1d) and the pericholangial fibrogenesis (Fig. 1f) were frequently encountered in these cases.

In the published evidence concerning hepatic capillariasis in fish or other vertebrate hosts, no biliary lesions were reported to date. Kutzer and Otte (4) have mentioned occasional signs of icterus in liver specimens of some infected fish species, but details of associated pathological lesions have not been reported. Liver fibrosis induced by hepatic capillariasis is well documented only in mammalian hosts (9, 5, 3), including the experimental septal fibrosis reported in rats as a consistent and reproducible liver lesion after infection with *Capillaria hepatica* (1, 7, 10).

Our evidence of cholangiofibrosis in the Mediterranean barbel harbouring hepatic capillariasis, may be a consequence of a non-specific reaction to parasitic chronic injury, even though an independent pathogenic mechanism cannot be ruled out.

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THE MECHANICAL TRANSMISSION OF *Trypanosoma evansi* BY *Haematobia minuta* (DIPTERA: Muscidae) AND *Hippobosca camelina* (DIPTERA: Hippoboscidae) FROM AN INFECTED CAMEL TO A MOUSE AND THE SURVIVAL OF TRYPANOSOMES IN FLY MOUTHPARTS AND GUT

(A Preliminary Record)

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ABSTRACT

The role of *Haematobia minuta* Linnaeus and *Hippobosca camelina* Leach in the transmission of camel trypanosomiasis was assessed. In a preliminary survey, there were no trypanosomes in the mouth parts or gut of the field collected, *H. minuta* and *H. camelina,* following dissections. *H. minuta* mechanically transmitted *T. evansi* from an infected camel to mice but *H. camelina* failed to do so. Results show that *H. minuta* may play just a minor role in the transmission of camel trypanosomiasis as no parasites were found in the mouth parts and gut of the field collected flies and the transmission rate was low. Although parasites survived in the gut of both flies for as long as thirty minutes, the survival of trypanosomes in the mouth parts of both flies was restrictive as far as mechanical transmission is concerned. *H. camelina* failed to transmit *T. evansi* experimentally and therefore its importance in the transmission of camel trypanosomiasis in Northern Kenya was ruled out, unless by regurgitation. Sub-inoculated homogenates of flies previously fed on an infected camel were infective to Balb C mice but the significance of this in relation to mechanical transmission in nature remains unclear.

Key words: biting flies; camel trypanosomiasis; *Haematobia minuta; Hippobosca camelina;* Northern Kenya; *Trypanosoma evansi*

INTRODUCTION

A number of biting flies have been implicated in the mechanical transmission of *T. evansi* (25). Several researchers have reported mechanical transmission of trypanosomes by stable flies (1, 2, 8, 9, 10) and tabanids (14). Oyieke (15) showed that tabanids and *Stomoxys* could mechanically transmit camel trypanosomiasis *(T. evansi*) in Northern Kenya.

The Kenyan camel population is concentrated in Northern Kenya. Camels form the economic mainstay of the nomadic pastoralists in the region. Camel milk, meat and blood are sources of protein. All manner of transport is provided by the camel with camel hide and meat forming a possible source of income. The camel is also important for social functions such as marriages as it is included in bride prices. *T. evansi* infection has been reported to be a serious constraint on camel production (16, 20) in Northern Kenya.

H. minuta and *H. camelina* are predominant camel ectoparasites in this region but their role in the transmission of camel trypanosomiasis in the tse-tse free Northern Kenya has not been evaluated. Further more direct blood feeding effects of both flies can lead to anaemia, weight loss, losses in milk and meat yields and even death (13).

Members of the genus *Haematobia* (syn. *Lyperosia*) belong to the subfamily Stomoxyinae along with Stomoxys. Haematobia, commonly referred to as horn flies, closely resemble *Stomoxys* but are much reduced in size and are brown in colour. The body length of *Haematobia minuta* is three to four millimetres (mean length 3.54 ± 1.6 mm) and unlike *Stomoxys,* the mouthparts are ensheathed by palpi which are as long as the proboscis. The antennal *arista* is simple with hairs projecting from one side only as in *Stomoxys* spp. The wing of *H. minuta* is similar in structure to that of *Stomoxys* but is greatly reduced measuring 2.5 mm (mean length of 2.43 ± 0.2 mm). *H. minuta* can be distinguished from *Stomoxys* spp. based on size and the length of palpi in relation to the proboscis. Palpi of *Stomoxys* are extremely slender and short and cannot be seen when viewed

from above. *Haematobia* on the other hand have palpi that are flattened from side to side and form a complete sheath for the proboscis which they almost equal in length (22).

Hippobosca camelina belongs to the family Hippoboscidae. Adults of this family are flattened in appearance, with wings which are either well developed, shortened or absent. The legs are short and strong and broadly separated. The adults are external parasites of birds and mammals and range in size from 2.5 to 10 millimetres. Members of this family are larviparous and breed independent of water. Hippoboscids of mammals comprise four genera: *Melophagus, Lippottena, Neolipottena* and *Hippobosca.*

Hippobosca camelina is commonly known as the camel fly. It is a large, stout, flattened brown fly that measures 9 to 12 millimetres in body length. The proboscis (0.6 to1.2 mm) is extremely slender and protrusible and is composed of *labella, hypopharynx, labrum, epipharynx, labium* and *rostrum.* The tip of the *labella* is armed with chitinous teeth to pierce the host skin. The wing (8 to 10 millimetres) is well developed and clear with solid radial vertical veins. The thorax of this fly is characterized by a pattern of light and dark regions. The legs are hairy and tarsi have claws which enable the insect to cling to the hair or feathers of the host. *H. camelina* is commonly found in areas populated by camels such as India, Arabia, North and East Africa (4).

MATERIALS AND METHODS

Study area

The study area covered 23,000 square kilometres including the Chalbi desert and its surrounding watersheds. It lies between 1° 50´ and 3° 3´ north and 35° 00´ east (6). The highest point in the area is Mount Kulal an altitude of 2,500 metres which supports an evergreen mist forest. The Marsabit Mountains lie to the east. To the extreme north are the Hurri Hills and to the south east border the Ndoto, Mara and Nyiru Mountains.

Between the mountains and Chalbi desert lie a series of climatic zones with associated vegetation communities and soil types as described by Herlocker (3). With the exception of Mount Kulal, the area receives very low rainfall (100—300 millimetres *per annum*), high evaporation and an altitude of 1,000 metres. Open surface water is rare and there are no reliable watering sources except for the springs around the Marsabit escarpment. Dwarf shrubs (*Indigofera spinosa, Duosperma eremophilium, Sericocomopsis hildebrandti, Salsols* and *Suedda* species) and low density annual grasses, shrubs and *Acacia* species bushland are the commonest vegetation in the area. The sand plains between Mount Kulal and Mount Marsabit contain various vegetation communities dominated by *Acacia reficiens, A. sengal,* and *A. nubica* with *Aristidia* and *Cenchrus* species as the commonest grasses. The rest of the area is completely devoid of plants. The area is hot and the mean monthly temperatures range from thirty to forty-seven Celsius and a minimum of eleven to twenty-six Celsius.

About 30,000 nomadic pastoralists live in the area. They are mainly of the Gabra, Rendille Borana and Samburu ethnic groups. The people in the area own about 500,000 head of livestock (shoats and camels). Other animals (wildlife) in the area include zebra, oryx, hyena, giraffe, gazelle, leopard, gerenuk, wildcat, hare, and caracal (6).

There is a long (March—May) and a short (November to December) rainy season. January and Febraury and June to October are the dry seasons. The specific site at which this study was conducted is Ngurunit.

Dissections of field collected flies

As a preliminary survey, this exercise was undertaken to ascertain if randomly collected *H. minuta* and *H. camelina* had trypanosomes in the mouth parts and/or gut. Flies collected using sweep nets were placed individually into Kilner jars containing cotton wool soaked in ether to immobolize them. Each fly mouth part and gut was dissected on a microscope slide containing a drop of 10 % foetal calf serum in physiological saline and viewed on a portable field microscope (Kyowa optical 711340). A total of 200 dissections were carried out.

Transmission trails

Before the transmission trials flies were captured using the standard insect sweep nets and kept in vials. Flies were classified into two types based on their feeding history: (1) wild caught but fed on camel blood a day before experimentation; (2) freshly obtained from the animal hosts. Before trials, the mouth parts of the flies and their gut were dissected from a random sample of fifty flies to ensure the absence of infection. No trypanosomes were detected. The donor camel host was naturally infected with *T. evansi* following a preliminary survey. Trials were conducted when parasitaemia in the donor was 10^5. ml^{-1}. Studies were carried out with flies following the same protocols used in Ogonji (12). Each of the 200 *H. minuta* and 200 *H. camelina* was held in a small plastic vial with one end consisting of netting. The fly was allowed to take a partial meal from the camel belly. Feeding was interrupted and the fly transferred to an uninfected mouse to complete its meal. Parasitaemia in the 400 mice was monitored for sixty days as described below. In total one hundred trials were attempted.

Parasite survival in fly mouth parts and gut

To determine how long trypanosomes could be detected in *H. minuta,* flies were fed on a camel infected with *T. evansi,* flies were dissected after the following time intervals: 1, 2, 3, 4, 5, 10, 15, and 20 minutes and their mouth parts examined for trypanosomes (n = 50 for each time interval). In order to evaluate the persistence of the parasites in the gut, ten flies were killed at these five intervals (15, 30, 45, 60, and 75 minutes), and their mid guts examined for trypanosomes (n = 50 for each time interval).

Innoculation of whole fly homogenate into mice. To confirm the infectiousness of parasites to mice after ingestion by the fly, flies were fed on an infected camel and killed at ten intervals (10, 20, 30, 60, 100, 150, 180, 210, 240, and 300 minutes). Individual flies at each interval were homogenised in 0.5 ml normal saline and the resulting mixture inoculated into uninfected mice. Infection by each mixture was attempted twenty times (two mice per trial). Parasitaemia was monitored for sixty days by microscopic examination of wet smears prepared from blood drawn from the tail vein of the mouse.

RESULTS

No trypanosomes were found in the mouth parts or gut of the randomly field collected *H. minuta* and *H. camelina.*

The results presented on Table 1 show that a minimal level of transmission was achieved with flies of both feeding histories. Flies previously starved had a slightly higher transmission rate than previously fed flies but the differences were not significant ($\chi^2 = 1.46$, $\alpha = 0.05$, $\rho = 0.321$). *Hippobosca camelina* failed to transmit *T. evansi* mechanically. *H. minuta* transmitted *T. evansi* (2 of 100 blood fed; 3 of 100 unfed).

Table 1. Frequency of the mechanical transmission of *Trypanosoma evansi* to Balb C mice following interrupted feeding on an infected camel

Fly feeding history	Trials	% successful transmission
H. minuta		
Blood fed	100	2
Unfed (starved)	100	3
H. camelina		
Blood fed	100	0
Unfed (starved)	100	0

Motile trypanosomes were seen in the proboscis of both flies only for up to five minutes (Table 2) after the infective blood meal. Although a higher number of parasites were seen in the proboscis of *H. minuta* than in *H. camelina,* the differences were not significant ($\chi^2 = 3.8$, $\alpha = 0.05$, $\rho = 0.19$).

The survival of parasites in the gut was determined by dissecting a total of 350 *H. minuta* and 350 *H. camelina.* Parasites were active and motile up to ninety and seventy-five minutes

Table 2. Trypanosomes in the proboscis of flies at various time intervals following a blood meal on an infected camel

Time (min)	*H. minuta*	*H. camelina*
1	9	7
2	7	3
3	2	0
5	0	0
10	0	0
15	0	0
20	0	0
25	0	0

In the guts of *H. minuta* and *H. camelina* respectively as shown on Table 3. There was no evidence of live trypanosomes two hours after the infective blood meal. The differences in the survival time-span in the two flies were not significant ($\chi^2 = 3.0$, $\alpha = 0.05$, $\rho = 0.61$).

Sub-inoculation of whole fly homogenates into mice (Table 3) revealed that parasites remained infective up to sixty minutes in *H. minuta* and up to thirty minutes in *H. camelina. T. evansi* grew in Balb C mice with a prepatent periods of four to five days. Mice that were infected eventually died by day 12—13.

Table 3. Trypanosomes in the midgut of flies at various time intervals following a blood meal on an infected camel

Time (min)	*H. minuta*	*H. camelina*
15	+++	+++
30	+++	+++
45	+++	+++
60	++	++
75	++	+
90	+	–
120	–	–

Legend: +++ — parasites very active; ++ — parasites motile; – — no motile parasites

DISCUSSION

The failure of *H. camelina* to transmit *T. evansi* is not entirely a unigue phenomenon to this species; *Aedes* spp. (19) also failed to transmit *T. evansi* mechanically.

The mechanical transmission of *T. evansi* by *H. minuta* has been demonstrated, but the rate of transmission of 3% is relatively low compared to other biting flies such as tabanids – Oyieke (15) and stable flies (up to 25%) – Mihok *et al.* (7). The *T. evansi* that was transmitted readily was not an African strain but from a capybara in South America. It is however interesting to note that transmission was similarly low when the parasite was from a camel (1 in 110 attempts) according to reports by Mihok *et al.* (8). Differences in the transmissibility of *T. evansi* from different regions of the world is a factor that requires further and more detailed investigations. The mechanical mode of transmission occurs through either the contamination of mouth parts or regurgitation of gut contents (5).

Parasite survival in fly mouth parts is a major requirement for mechanical transmission. *T. evansi* survived in the mouth parts of both flies for very short periods of time (two minutes in *H. camelina* and three minutes in *H. minuta*). Prolonged longevity (seven minutes) of *T. evansi* have been reported in mouth parts of *Stomoxys* (18) and tabanids (15). The retention of viable trypanosomes may be attributed to morphological and physiological features of fly mouth parts. Tabanids with larger and fleshier *labella* (11) are likely to retain more parasites than *H. minuta* and *H. camelina.* The probosces of both *H. minuta* and *H. camelina* are extremely slender compared to those of stable flies and tabanids. The rapid disappearance of parasites from the mouth parts of *H. minuta* and *H. camelina* and the lack of intermittent feeding pattern reduces the chances their chances to transmit trypanosomes mechanically in nature.

Based on results of this study, the gut environment of both flies appeared to have been more conductive for the survival of *T. evansi* compared to the mouth parts. Live trypanosomes were detected up to ninety minutes after an infective blood meal. The prolonged survival of the parasites in the gut is further supported by results in Table 4, which show that there were infective trypanosomes in the gut of both flies up to thirty minutes after the infective blood meal. The regurgitation of viable trypanosomes, reported in *Stomoxys calcitrans* by Straif *et al.* (17), is therefore a possible mode of transmission of *T. evansi* by both *H. minuta* and *H. camelina.* It is not known if these flies regurgitate during normal feeding although the phenomenon is known to occur when *Stomoxys calcitrans* is fed artificially on fluids in capillary tubes (5, 17).

Table 4. The infectivity of trypanosomes in flies following the sub-inoculation of homogenates into mice at various time intervals

Time (min)	*H. minuta*	*H. camelina*
10	++	++
20	++	++
30	++	++
60	+–	–
100	–	–
150	–	–
180	–	–

Key: ++ — both mice infected; +– — one mouse infected; – — neither mouse infected

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STUDIES ON THE GENUS *Cotylophoron Fischoeder,* 1901 (Paramphistomidae), RECOVERED FROM NIGERIAN CATTLE

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ABSTRACT

Four species of the genus *Cotylophoron* Fischoeder, 1901, namely *Cotylophoron cotylophorum, Cotylophoron fuelleborni, Cotylophoron indicum* and *Cotylophoron jacksoni* were recovered from the inner walls of stomachs of cattle slaughtered in abattoirs in the North and South of Nigeria. Some parasites were flattened and diagnostic features taken while some were sectioned in the median saggital plane from where the histology and morphology of the pharynx, genital atrium, acetabulum and testes were analysed for identification. Photomicrographs of the features used in identifications were made and are presented here. No significant damage was done to the host tissues. Parasite loads ranged between 20 and 700 parasites in the infected animals and prevalence was 35 % for *C. cotylophorum,* 2 % for *C. fuelleborni,* and 10 % for *C. indicum,* 0.2 % for *C. jacksoni. C. fuelleborni, C. indicum,* and *C. jacksoni* are being reported in Nigeria for the first time.

Key words: acetabulum; *Cotylophoron* spp. (Paramphistomidae); genital atrium; pharynx; trematodes

INTRODUCTION

Parasites of the genus *Cotylophoron* are trematodes of the family Paramphistomidae Fischoeder, 1901 and are parasitic in the alimentary canal of many ruminants. Mature parasites are especially prevalent in the reticulum and rumen where they, after Horak (11), rarely produce clinical symptoms. Immature migrating parasites have been reported causing serious disease and even the death of their hosts by burying themselves in the submucosa of the duodenum and feeding on the epithelial cells of Brunner's gland which results in anorexia, polydypsia, profuse foetid diarrhoea, a drop in plasma protein concentration and anaemia (2, 3, 11, 12, 23, 17).

Whilst various paramphistomes have been identified as aetiological agents of paramphistomiasis, the only extensively species studied in this genus is *Cotylophoron cotylophorum* (11, 23, 18). The documentation of prevalence and pathogenicity can only be ascertained for species that have been accurately identified. Owing to the difficulties in identifying members of this group except through the median saggittal sections there has been confusion in the taxonomy of this group (15, 4, 19, 5, 6, 7). Näsmark (15) and Round (19) point out that the material referred to as *Cotylophoron* sp. by earlier workers was in fact found to be *Paramphistomum microbothrium.* Schillhorn Van Veen *et al.* (20) listed *Cotylophoron cotylophorum* as present in past collections of parasites obtained from Nigeria.

The aim of this study was to create descriptive studies for the genus *Cotylophoron* in an attempt to establish the frequency and spectrum of species in Nigeria as among them are well known pathogens. Such information according to Rolfe *et al. (18) would be valuable in designing control measures that are efficient and economical.*

MATERIALS AND METHODS

Specimens were obtained from the inner walls of the rumen and reticulum from cattle slaughtered in the abattoirs in Benin in the South and Zaria in the North of Nigeria. The parasites were collected in plastic containers containing normal

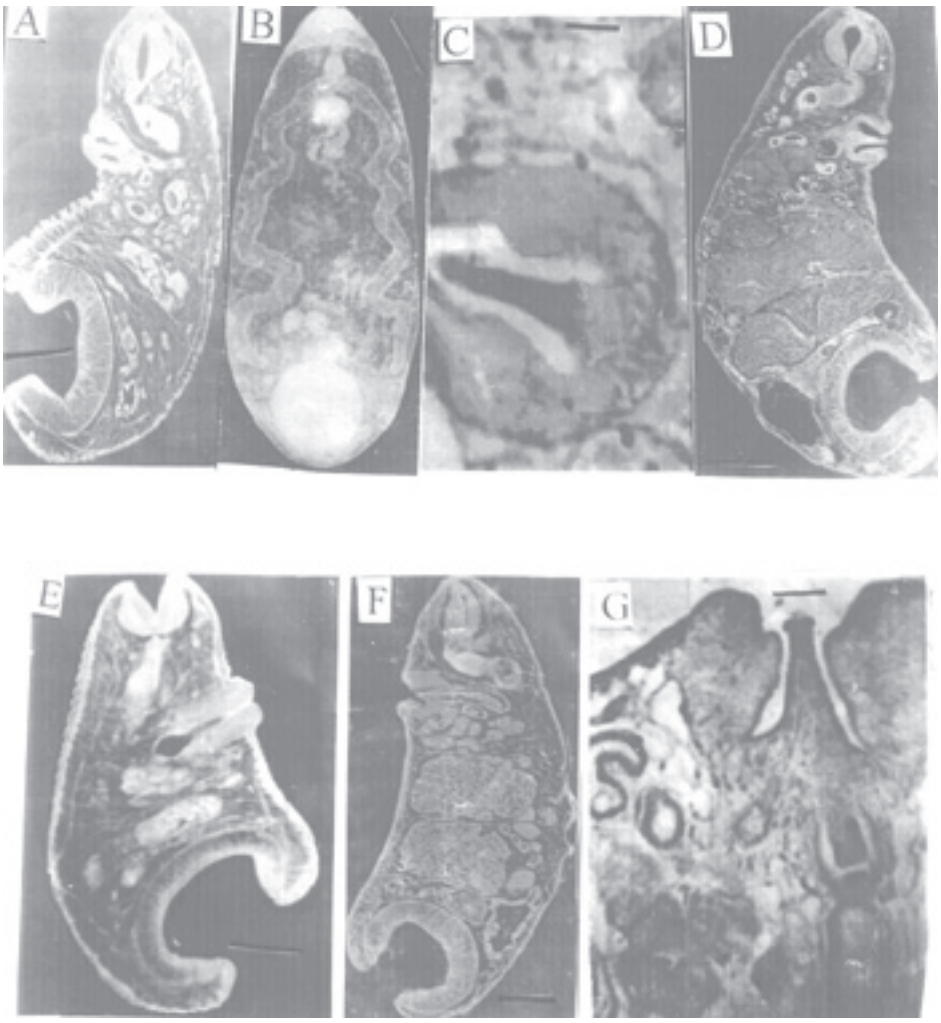

Fig. 1. Genus *Cotylophoron* Fischoeder, 1901

A) *C. cotylophorum* median saggital section of whole worm, note the terminal acetabulum, genital sucker of genital atrium and thick integument (Scale bar — 1.5 mm); B) *C. colytophorum* flattened whole worm ventral view note the ovary and Mehlis gland above the acetabulum, the gut caeca and genital atrium at the ceacal bifurcation (Scale bar — 2 mm); C) *C. cotylophoron* genital atrium with a clearly marked sucker (Scale bar — 200 mm); D) *C. jacksoni* median sagittal section of whole worm note the terminal acetabulum with genital atrium of cotylophoron type, the large lobulated testis and the *pars prostatica* (Scale bar — 1 mm); E) *C. fuelleborni* median sagital section of whole worm, note the genital sucker and relatively large acetabulum (Scale bar — 1 mm). F) *C. indicum* median sagital section of whole worm: note the genital sucker round testis and excretory bladder above the terminal acetabulum (Scale bar — 1 mm); G) *C. indicum* median sagital section: note the strongly developed sucker, the *pars prostatica* and *pars musculosa* (Scale bar — 200 mm)

saline and were washed in the same solution. Some specimens were teased out for egg measurements; some were flattened dorso-ventrally between two slides to facilitate the examination of diagnostic features (vitelline glands, position of testes, oesophagus, nature of caeca and uterus). Some specimens were fixed and preserved in formol saline and 70 % ethanol for a histological characterisation in the median sagittal sections using the systems proposed by Näsmark (15) and Yama-guti (24) in which the acetabulum, pharynx and genital atrium were analysed. The specimens were prepared for sectioning by the method of Mahoney (13). Brief graded alcohol series were used for dehydration of specimens, which were then embedded in wax, sectioned with a rotary microtome and stained using haematoxylin-eosin. The specimens were mounted in Canada balsam. The slides were dried on a hot plate at 60 °C for ninety-six hours. Photographs were taken using a camera mounted on a microscope while diagnostic measurements were taken with calibrated microscope.

RESULTS

Cotylophoron cotylophorum Fischoeder, 1901

Description:

The body is conical (Figs. 1A and B). The width is large in relation to body length. The widest part is in the region just above the acetabulum. When fresh they

Table 1. Measurements for flattened specimens and median sagittal sections in millimeters for *Cotylophoron* species from Nigeria

	Median section *C. cotylophorum*	Flattened *C. cotylophorum*	Median section *C. fuelleborni*	Flattened *C. fuelleborni*	Median section *C. indicum*	Flattened *C. indicum*	Median section *C. jacksoni*
Body length	4.27 ± 0.28	10.75 ± 1.29	2.86 ± 0.70	4.849 ± 0.45	5.9 ± 0.42	10.2 ± 0.75	7.6 ± 1.29
Body breadth	2.33 ± 0.15	4.1 ± 0.39	1.70 ± 0.27	2.68 ± 0.15	2.43 ± 0.10	3.92 ± 0.33	3.04 ± 0.44
Acetabulum diameter	1.52 ± 0.09	2.04 ± 0.29	1.38 ± 0.16	1.63 ± 0.14	1.64 ± 0.10	2.00 ± 0.11	1.83 ± 0.32
Ratio of acetabulum diameter to body length	1:2.83 ± 0.31	1:4.34 ± 0.55	1:2.16 ± 0.63	1:3.0 ± 0.38	1:3.62 ± 0.10	1:5.13 ± 0.49	1:4.2 ± 0.57
Pharynx length	0.54 ± 0.05	0.62 ± 0.10	0.42	0.47 ± 0.06	0.71 ± 0.10	0.79 ± 0.07	0.73 ± 0.01
Ratio of pharynx length to body length	1:7.98 ± 0.71	1:14.41 ± 3.48	1:6.90 ± 1.56	1:10.52 ± 0.80	1:8.41 ± 0.10	1:12.88 ± 1.38	1:10.54 ± 1.84
Oesophagus length	0.57 ± 0.12	0.62 ± 0.10	0.32 ± 0.3	0.31 ± 0.06	0.72 ± 0.29	*	0.96 ± 0.25
Anterior testis length	1.25 ± 0.23	1.04 ± 0.38	0.46 ± 0.12	0.46 ± 0.06	0.19 ± 0.10	2.54 ± 0.41	2.32 ± 0.73
Anterior testis breadth	0.31 ± 0.23	0.91 ± 0.31	0.19 ± 0.05	0.39 ± 0.04	0.86 ± 0.10	2.52 ± 0.41	1.10 ± 0.30
Posterior testis length	1.25 ± 0.23	1.01 ± 0.34	0.46 ± 0.12	0.47 ± 0.07	0.19 ± 0.10	2.64 ± 0.43	2.32 ± 0.73
Posterior testis breadth	0.31 ± 0.23	0.94 ± 0.35	0.19 ± 0.05	0.40 ± 0.05	0.86 ± 0.10	2.64 ± 0.43	1.10 ± 0.3
Genital atrium diameter	0.57 ± 0.04	0.84 ± 0.03	0.44 ± 0.08	0.66 ± 0.04	0.64 ± 0.11	0.98 ± 0.14	0.73 ± 0.01
Ovary diameter	0.16 ± 0.01	0.26 ± 0.09	0.20 ± 0.09	*	0.32 ± 0.05	0.55 ± 0.10	0.52 ± 0.02
Egg size		0.059 x 0.102		*		0.063 x 0.119	0.52 x 0.132

* — Data not obtained

Table 2. Distribution, prevalence and acetabulum muscle units numbers of *Cotylophoron* species in Nigerian cattle

Species recovered	Geographical area where found	Habitat	%Cattle infected	Damage to host tissue	Number of parasites range	de*	di*	vi*	ve*
C. cotylophorum	Benin	Rumen & Reticulum	35	slight	20—700	19	41	49	17
C. fuelleborni	Benin	Rumen & Reticulum	2	slight	20—250	15	42	44	16
C. indicum	Benin	Reticulum	10	slight	20—300	20	40	46	14
C. jacksoni	*Zaria*	*Rumen & Reticulum*	*0.2*	*slight*	*10—40*	*15*	*39*	*34*	*13*

de — dorsal exterior circular muscles units; di* — dorsal interior circular muscles units*
vi — ventral interior circular muscles units; ve* — ventral exterior circular muscles units*

are yellowish brown or pink in color. The integument is thin and marked by well-defined transverse wrinkles. The acetabulum is subventral and its measurements and those of other organs are given in Table 1.

The acetabulum is of the *Cotylophoron* type. In median sagittal sections the dorsal exterior muscles units *(de)* are in a single row. The units are largest at the terminal of the row and then they decrease progressively. The dorsal interior muscle units *(di)* and ventral interior muscle units *(vi)* are sometimes numerically the same. The ventral exterior muscle units *(ve)* follows the same pattern of development as the dorsal exterior muscle units. The dorsal exterior and ventral exterior muscle units are distinguished from the radial layer by obliquely running longitudinal branched muscle bands. In the dorso-ventral direction the acetabulum sometimes gives a semicircular shape, which is occasionally surrounded by a fold. The number of units in the four muscle series is given in Table 2.

The pharynx is terminal and is of the *Paramphistomum* type. The interior circular layer has oblong units, which are quite developed, and extend the whole length of the

pharynx. The interior longitudinal layer occupies one fourth of the width of the pharynx. The exterior limit is not distinct; the median circular is absent. The radial layer is present but not well developed. The exterior circular layer is present and the units are grouped into small packs along the length of the pharynx. The exterior longitudinal layer is present and small. The posterior, anterior and lip-sphincters are absent. The basal circular layer is well developed and divided into two rows, the external layer being smaller than the interior layer. The oesophagus has a bulbous expansion where it joins the caeca; it is made of two thin muscle layers which expand to form the bulb. A thick integument-like layer lines its lumen.

The gut caeca run laterally forming about six bends and terminate on top of the acetabulum and the blind ends each are directed towards the ventral side. The testes are deeply lobed and diagonally placed. The uterus runs in the midline and makes a few irregular curves before leading into the metatherm, which is straight. The vitelline glands are scattered extending from the level of the gut bifurcation to the acetabulum. They lie between the lateral margins of the body and the gut caeca. They ovary is round. The Mehlis gland lies ventrally to the ovary in median sagittal sections and side-by-side in the flattened specimens.

The genital atrium (Fig. 1 C) is situated below the caecal bifurcation. It is of the *Cotylophoron* type. The genital folds form a muscular genital sucker, which is delimited from the parenchyma of the body. The genital papilla rises from the bottom of the genital atrium. There is no ventral atrium. *The ductus ejaculatorius* and *metatherm* open side by side at the tip of the genital papilla. The *pars prostatica* is cylindrical and short. The *pars musculosa* makes one loop and then leads into the *vesicula seminalis,* which makes a few loops before branching into two *vasa deferentia,* which lead to the posterior and exterior testes. Laurer's canal and the excretory canal cross before opening separately to the exterior. The excretory bladder is made of a gland-like tissue, which has an irregular thickness. The eggs are operculate and light green with scattered granules in the yolk.

C. fuelleborni Näsmark, 1937

Description:

The body is conical (Fig. 1 E). The widest part is just above the acetabulum. When fresh they are yellowish white. The integument is slightly wrinkled and thick. The acetabulum diameter is large compared with the short body length. Its measurements and those of other diagnostic organs are given in Table 1 for median sections and for flattened specimens. It is of the *Cotylophoron* type (Fig. 1 E) and has no fold surround it. It follows the description for *C. cotylophorum.* The number of muscle units in the four series is given in Table 2. The pharynx is located terminally and is of the *Paramphistomum* type and follows the description for *C. cotylophorum.* The oesophagus has no bulbous expansion. It consists of two layers; the outer is longitudinal while the inner is circular. The gut caeca run laterally forming few beds and terminates in the middle of the acetabulum. The blind ends are directed to the ventral side. The testes are slightly lobed and diagonally placed. The uterus is wavy and runs in the midline behind the testis. The vitelline glands extend from the caecal bifurcation to the acetabulum along the lateral margins of the body. The ovary is adjacent to the Mehlis gland and both lie between the posterior testis and the acetabulum. The genital atrium, the *ductus ejaculatorius* and *metatherm* resemble those described for *C. cotylophorum.* The *pars prostatica* is round and leads to the *pars musculosa,* which has only one loop. The *pars musculosa* leads to the *vesicula seminalis,* which makes a few loops and then branches into two vasa *deferentia* leading to the posterior and anterior tests. Laurers's canal crosses the excretory canal before opening to the exterior. The excretory bladder is made of a gland-like tissue with an irregular thickness.

C. indicum Stiles et Goldberger, 1910

Description:

The body is conical (Fig. 1 F). The width is small in relation to the body length. The widest part of the body is around the posterior testis. When fresh they are yellowish-white in color. In most specimens the thin integument is not wrinkled. The measurements of the diagnostic organs are given in Table 1. The acetabulum is of the *Cotylophoron* type resembling the description given for *C. cotylophorum.* The number of units in the four series is given in Table 2. The pharynx is located terminally, and is of the *Paramphistomum* type resembling the description given for the pharynx of *C. cotylophorum.* The oesophagus has no bulbous expansion. It is made of two thin layers, which are uniformly thick. A thin integument-like layer lines the lumen of the oesophagus.

The gut caeca run laterally forming about six bends and terminate at the level of the acetabulum and the blind ends are directed towards the ventral side. The testes are slightly lobed and they lie in tandem. The entire shape of the testes is round. The *pars prostatica* is cylindrical and barrel shaped. It leads into the *ductus ejaculatorius* ventrally and *pars musculosa* dorsally. The *pars musculosa* makes a number of loops before branching into two *vasa deferentia* leading to the anterior and posterior testes. The metatherm and the *ductus ejaculatorius* open side by side at the tip of the genital papilla. The genital atrium is of the *Cotylophoron* type described for *C. cotylophorum* (Fig. 1 C). The excretory bladder is oblong and situated between the posterior testis and the acetabulum towards the dorsal side of the body. Laurer's canal crosses the excretory canal before opening to the exterior. The uterus is wavy and runs in midline behind the testes. The eggs are operculate and light green with scattered granules in the yolk.

***C. jacksoni* Näsmark, 1937**

Description:

The body is conical (Fig. 1D). The widest part is just above the acetabulum. When fresh they are yellowish-white in colour. The tegument is thin and has transverse wrinkles, which are not very pronounced. The acetabulum is of the *Cotylophoron* type previously described for *C. cotylophorum.* The number of muscle units in the four series is given in Table 2. The pharynx is located terminally and is of the *Paramphistomum* type previously described. The oesophagus has no bulb though the muscles close to caecal bifurcation are slightly thickened. The gut caeca run laterally forming six bends, the blind ends are directed ventrally at the sides of the acetabulum.

Clusters of vitelline glands extend from the pharynx to the acetabulum. They lie between the caeca and the lateral margins of the body. The testes are in tandem and deeply lobed. They lie very close to each other in the mid-third of the body. Their entire shape is oblong with longer sides lying dorso-ventrally. The genital atrium is of *Cotylophoron* type. The *ductus ejaculatorius* and *metatherm* open separately at the tip of the genital papilla. The ovary is oval and lies between the posterior testis and the acetabulum. The Mehlis gland lies ventral to the ovary. The excretory canal crosses Laurer's canal. The excretory bladder is round and is made of thin tissue. The *pars prostatica* is spherical, it is connected to the *pars musculosa,* which makes one loop and leads to the *vesicula seminalis,* which has very small lumen and makes several closely packed loops. The *vesicula seminalis* branches into two *vasa deferentia* which branch to the anterior and posterior testes. The uterus is wavy and runs dorsally to testes. It is situated along the midline of the body. The eggs are operculate and transparent with a few granules scattered in their yolk.

DISCUSSION

The described parasites belong to the genus *Cotylophoron* Stiles et Goldberger, 1910. The body shape, the presence of well-developed genital sucker, position and shape of testes, acetabulum, ovary, and vitelline glands agree with the descriptions of Närsmark (15) and Yamaguti (24).

The first parasite in the description is assigned to the species *C. cotylophorum* Fischoeder, 1901 because it possesses a well-developed oesophaegal bulb, which is characteristic of this species. The histological structures of the acetabulum, pharynx and genital atrium are similar between species. Even the genital atrium pointed out by Sey and Graber (21) to be different does not seem to show any difference from previous descriptions and illustrations of Näsmark (15) and Yamaguti (24). The diagonally placed testes differentiate this species from *C. indicum* and *C. jacksoni.* The ratio of the genital atrium to pharynx length used by Näsmark (15) to separate species shows a narrow range that it seems impractical to detect such small differences to determine species. Sometimes the action of preservatives may constrict the genital atrium and alter this ratio considerably.

C. fuelleborni Näsmark, 1937 was identified on the basis of it lacking an oesophageal bulb and the testes are diagonal like those of *C. cotylophorum.* The thick integument, the small body size with a mean of 2.08 mm and a large acetabulum with a diameter of 1.38 mm agree with the description of Näsmark (15) for this species.

C. indicum Stiles et Goldberger, 1901 was identified on the basis of the testes, which are in tandem, large and round. There is no oesophageal bulb and the integument is thin. The body is narrow and elongate. These features resemble the description of Näsmark (15) for this species.

C. jacksoni Näsmark, 1937 was identified on the basis of the testes that are in tandem, large, deeply lobed, oblong and lie close to each other. It has a slightly bulbous oesophagus and attains the largest size among members of this genus. The above features correspond to the description of Näsmark (15) and Yamaguti (24) for this species. The parasites classified as *C. fuelleborni, C. indicum* and *C. jacksoni* are being reported in Nigeria for the first time.

While the parasites described for the first time in Nigeria have not been assayed for their involvement in disease it should be noted that these parasites were previously reported in Kenya, Sudan and Congo – Näsmark (15) and Round (19). Their occurrence in Nigeria has the following implications: firstly that these parasites have always been present in Nigeria but no one had come across them in their study, and secondly the parasites were introduced into Nigeria by the movement of cattle across borders and eventually now been established here. There is a probability that they could be infecting the same snails as *Cotylophoron cotylophorum* in which case they could affect its development in the snail synergistically or antagonistically as it was demonstrated for *Paramphistomum daubneyi* and *Fasciola hepatica* – Abrous and Dreyfuss (1).

Our projection is that the histological methods as employed in the current study should form a base on which molecular techniques which are now frequently used in the taxonomy of organisms should be established for paramphistomes in general. Pillay and Pillay (16) have employed RAPD in distinguishing between *Schistosoma* isolates that belong to the same class as *Cotylophoron.* Many recommendations on the utilization of molecular methods such as RAPD, RFLP, analysis of rDNA ITS regions, isozyme analysis, in phylogenetic studies have been suggested (22, 8, 14). RFLP backed by histological methods has been successfully used in the studies of *Trichostrongylus columriformis* and *Oncomelania hupensis* – Grant and Whitington (9) and Hope and McManus (1)). This access is attractive in that in areas where it has been applied it has enabled reliable separation of

organisms to species level. The only disadvantage of this method is that distinctions can be observed at subspecies level, which are not taxonomically valid (14).

Methods such as isozyme analysis, ITS of rDNA, RFLP, and RAPD have a disadvantage in that they cannot be used to study most previous collections of parasites, particularly those preserved in formalin because the DNA and proteins are permanently damaged to be of any use for studies with these methods. This makes comparison between current collections and previously studied material, using molecular methods, not possible for the time being perhaps until new methods are developed. It is our recommendation that ethanol be made the first choice material for preservation of specimens to enable such materials to be reviewed and verified in future by others using the molecular techniques mentioned above if need be.

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SPONTANEOUS KITTEN'S CYCLOPIA
(A Case Report)

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ABSTRACT

A case of a congenital cyclopia was studied from the anatomical point of view and described in the present report. The purpose of this study was to approach the causal agent and the timing of this malformation. A possible teratogenic agent which caused this cyclopia could be a chemical substance in the beginning of organogenesis. This case which belongs to the group of "cyclopic congenital malformations" was discussed in the frame of the literature already existing.

Key words: cat; congenital malformation; cyclopia; kitten; teratogenesis

INTRODUCTION

Cyclopia is a simple type of grave congenital malformation. This malformation is spectacular and belongs to the group of "cyclocephalic malformations". This group is characterized by various gradations of craniofacial dysmorphism usually with mandibular, maxillary, ocular and nasal defects (16, 13). A single orbit with a single or two fused ocular bulbs is the principal morphological feature of cyclopia. The absence of the nose, olfactory nerves, bones of the median part of the facial skull, a reduced forebrain with a single brain ventricle, and a proboscis above the eye are typical characteristics of cyclopean "monsters".

Using the ocular bulbs as a criterion, five types of cyclopia could be recognized and these types vary from *synophthalmia*, where two ocular bulbs are situated closer to the midline than it happens under the normal conditions, to *cyclopia perfecta,* where only one ocular bulb is present (10).

Little information about cyclopia in cats is available in the relevant international literature. In one case described by Scott et al. (23), the kittens of three cats which were treated with griseofulvin presented cyclopia or anophthalmia with the absence of optic nerves and rudimentary optic tracts.

In the present article, a case of a cyclopic kitten, born alive, from a stray cat is described and the possible reasons as well as the occurrence of this malformation in other animals are discussed.

MATERIAL AND METHODS

The above mentioned kitten was euthanized by intracardial infusion of pentobarbital; its entire body was fixed in formaline (10%) and examined for external malformations. Radiographs of the head and the entire body for the evaluation of any skeletal deformities were taken. The skin as well as the soft tissues of the head and body were removed. The head was cut off, cleared and stained with the double staining technique of "Alcian Blue and Alizarine Red S" (33, modified by the authors). The head was then examined with the aid of a stereoscopic microscope. Subsequently, it was unroofed in order to examine the morphology of the brain. The single ocular bulb was removed and examined for any possible deviations from normal development.

RESULTS

The external examination, revealed an extensive malformation of the head, whereas the rest of the body appeared normal. One ocular bulb was prominent in the middle line of the facial division of the head. From the dorsal part of the ocular bulb a small rod-like structure ($\approx$3 mm in length) projected. The occipital, parietal, temporal lobes, were well developed and ossified. The

Fig. 1. Kitten with cyclopia perfecta

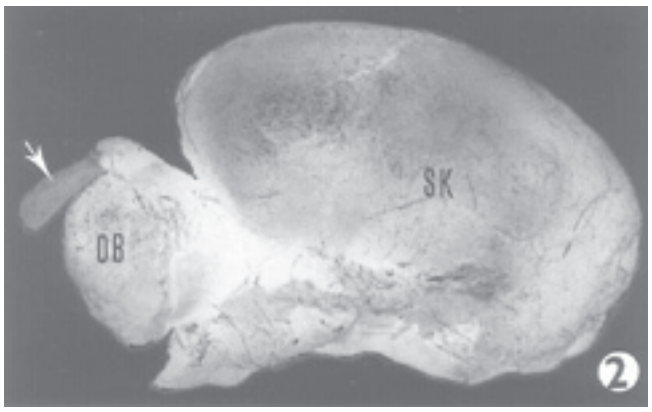

Fig. 2. Lateral view of the dissected skull (SK) of the kitten – the mandible is removed – with the single ocular bulb (OB) and the prominent rod-like structure *(arrow)*

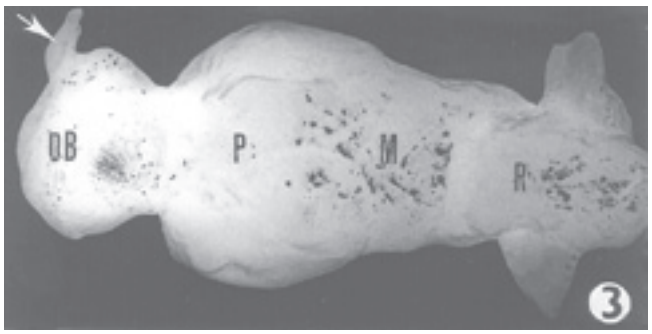

Fig. 3. Malformed brain – isolated – of the kitten (dorsal view) OB: ocular bulb, P: prosencephalon, M: mesencephalon, R: rhombencephalon, rod-like strucure *(arrow)*

caudal part of the frontal bone was normally developed, whereas the rostral part was incomplete and curved in towards the base of the skull. The zygomatic process of the frontal bone was ossified. The ethmoid, nasal, maxilla bones were completely missing; the zygomatic bone was found to be imperfectly developed, whereas the mandible was well developed and ossified. The tongue was normally developed.

The cerebral cortex appeared to be absent, whereas the rest of the cerebral hemispheres were rudimentary, fused and flattened. At the rostral edge, the two optic tracts converged in order to form a single optic nerve.

The ocular bulb, without the accessory structures, was enormous and well formed. After the dissection of the ocular bulb a single optic fovea was clearly recognised.

DISCUSSION

In an attempt to explain cyclopia, Speer (25) and Meckel (14) believed that this malformation arises through a more or less perfect fusion of two originally separate ocular bulbs and other concomitant fusions among various other head structures. Huscke (9) supposed that the ocular bulbs normally originate from a single median vesicle and that, in cyclopia, a developmental arrest causes the eye and forebrain to remain single. According to Adelmann (1), Willis (35) and Starck (26), this malformation is attributed to a defect directly in the anterior part of the notochord and the surrounding mesoderm. Rogers (20), Schowing (21) and Johnston *et al.* (11) attributed the malformation to a defect of the neural tube. Toerien (30) showed that extirpation of the anterior neural fold possibly leads to cyclopia. He concluded that this primary defect, causing a cyclopian monster, is a single defect of the anterior part of the neural plate, which leads to a reduction in forebrain development. When the anterior part of the neural crest is also involved, this results in the deficiency of the nasal septum, the anterior part of the sphenoid bone and the median bones of the face.

Thus, all the structures, which normally develop between the eyes, are absent resulting in fusion of the two eyes in one, which originally develop at the midline region. This view explains the numerous variations in the position of the ocular bulb in cyclopia, varying from two distinct eyes, to partially fused eyes and finally to a single midline eye. Similarly, anatomical variations of the optic nerves are thus found ranging from a single optic nerve (*cyclopia perfecta)* to two separate nerves,

fusing either within the retina or directly after leaving the eyes (synophthalmic embryos).

According to *Szabo* (28), there is no information on the genetic nature of cyclopia. On the other hand cyclopia can be produced experimentally in birds and lower vertebrates. Wolff (36) produced cyclopia perfecta by point-X-raying the region of head process in 18 to 22 hour chick embryos. De Jager (10) removed the anterior part of the cranial neural folds from chick embryos after 20—28 hours of incubation producing cyclopic and synophthalmic embryos.

A cyclopia-arrhinencephaly-otocephaly type of malformation occurred in mouse foetuses after maternal irradiation (15), treatment with vitamin A (8) and actinomycin D (27). Cyclopia was noted in rabbits, treated with methamphetamine hydrochloride (12), and in ferrets, treated with mustine hydrochloride (2).

Cyclopia was found and reported by Binns *et al.* (3) and Evans *et al.* (6) in certain stocks of range sheep in the United States. In fact, pregnant sheep which were fed with *Veratrum californicum* born lambs with cyclopia and other facial malformations. Analysis of the plant showed that three alkaloid substances were responsible, cyclopamine, cycloposine, and jervine.

The spontaneous occurrence of cyclopian malformations is rare. Palmer (18, 19) found one case of a cyclopic in 22 000 mice, one in 25 000 rats and eight cases in 10 000 rabbits. In 40 000 rabbits born over a period of 35 years, Nachtsheim (17) reported only one case of cyclopia.

In a survey of congenital defects of sheep in Australia, Dennis (5) found that about 5 % of the farmers had seen cyclopia in a large Merino population of lambs. In horses, five cases of cyclopia were noted during necropsy examination of approximately 9 000 foetuses or newborn foals (4). In swine of various breeds, Selby et al. (24) noted 16 cyclopic cases in 319 congenitally malformed piglets. Isolated cases of cyclopia have been reported in sheep and goats (29), humans (31) and foals (34). In cattle, a case of cyclopia was observed after a delayed pregnancy (7).

In the present case, we could not detect the teratogenic agent as well as the pathogenetic mechanism, since the kitten was born from a stray cat. Though, we can deduce it if we take into account the observations, the animal species and his ecology. This case could be characterized as cyclopia perfecta. The cat is a domesticated animal and is exposed to a contaminated environment with a lot of free embryotoxic agents. According to Schowing (22), many agents i.e. drugs, antimetabolites, solvents and pesticides cause congenital malformations in animals. Probably, the stray cat was fed with food or water, which contained such agents and especially solvents and pesticides. The timing of exposure of the embryo to a possible teratogenic agent could be considered approximately in the beginning of organogenesis, during the time when the eyes begin to be formed. The rod like structure which was projecting from the dorsal part of the ocular bulbus probably is a palpebral bud.

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CRUELTY TO ANIMALS AS AN UNLAWFUL ACTION

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ABSTRACT

Cruelty to animals is a problem that attracts the attention of both experts and the general public. Veterinary care authorities, too, are responsible for the protection of animals. They have the competence to bring proceedings against offenders. Actions associated with a greater degree of danger to society, such as cruelty to animals, fall within the competence of the penal system. Penal actions involving cruelty to animals that have been brought to the courts are relatively few because such actions can have repercussions and lack the effective participation of citizens, animal protection bodies, and ultimately the penal bodies. The present analysis indicates that the offenders are mostly males of low education coming from a socially poorer background. It should be stated that from the point of view of *de lege ferenda* it appears desirable to amend the existing legislation concerning the penal statute of cruelty to animals.

Key words: accused person; convicted offender; cruelty to animals; offence; offender; penal action; prosecuted person

INTRODUCTION

The efforts devoted to the adoption of legal regulations preventing cruelty to animals in The Slovak Republic are by no means a novelty (1, 4, 5). Unlawful actions associated with lower degree of risk to society are characterised as an offence while those associated with a higher risk are considered to be penal actions. The regulation of the observance of legal standards for the protection of animals as living beings against cruelty falls within the competence of veterinary care bodies. Penal action bodies have the competence to investigate and evaluate actions classified as penal offences (2).

The Act No. 488/2002 of the Civil Code of the Slovak Republic (Veterinary care act and amendments to some of the Acts) revoked the Act No. 115/1995 of the Civil Code, which constitutes a separate standard legal act that specifies the duties of subjects in relation to the prevention of cruelty to animals. The newly adopted Act, corresponding to EU law, includes legal regulations that bind the owners or keepers of animals to ensure protection and welfare of animals in their keeping. The Act prohibits cruelty to animals which is defined as all actions (with the exception of those related to justifiable health and approved experimental reasons), that cause damage to their health, impair parts of their bodies irreparably (except for sterilisation and castration), result in persistent or long-term behavioural disturbances that disrupt the biological capabilities of animals, cause unreasonable pain, injury, suffering, and that restrict the feeding and watering of animals thus impairing the state of their health. The Act defines other actions that are also prohibited.

Failure to respect the above mentioned restrictions is qualified as an offence that can be committed also by a person responsible for the observance of general rules set for conducting an experiment. The actions described above, but of greater violence, that meet the formal indicia, described as the facts of a case in § 203 of the Penal Code, are considered penal offences.

MATERIAL AND METHODS

The Act No. 140/1961 of the Penal Code in its wording of the later provisions allows the prosecution of a wrongdoer who has harmed an animal only for as a penal offence of damage to another person's property (§ 257 of the Penal Code). From the legal point of view, an animal is considered a subject or a thing that has an owner. However, judicial practice has dealt

only with those cases of intentional killing or injury to an animal in which the wrongdoer's action resulted in a loss to an owner of a more valuable animal or in which the expenses of veterinary care, related to the injury to an animal, reached a certain level. The manner of the offence and the level of brutality or cynicism could be considered only with regard to the severity of punishment if the case was brought to the court.

A completely different and new approach to the problems of preventing cruelty to animals is contained in the Penal Code amendment (Act No. 557/1991) which introduced into the penal law a new criminal offence "cruelty to animals". The offender may be a person who by his action(s) violates the rules of good behaviour and attention to an animal recognised by society with an intensity which means exposing an animal to cruel, inconsiderate treatment and unnecessary suffering regardless of the value of the animal as a living being.

After collecting data from official, generally available statistic surveys and particularly after obtaining and analysing documents with case law value, a review of the period of 1996—2001 was prepared concerning the following:

— persons prosecuted for cruelty to animals in SR (juveniles in particular);

— persons charged with cruelty to animals in SR (juveniles in particular);

— persons sentenced for cruelty to animals in SR (juveniles in particular);

— punishment inflicted on persons that were proven guilty of an offence classified as cruelty to animals.

The study of the collected documents allowed us to determine which animals were victims most frequently, which means and offensive objects the offenders used, and what were the consequences of such abuse of animals.

RESULTS

It was observed that in the period of 1996—2001 in The Slovak Republic altogether 73 persons were prosecuted for the criminal offence defined as cruelty to animals and of these 22 were juveniles (juvenile is a young person 15—18 years old). In the same period, 71 persons were indicted for this criminal offence and out of these 21 were juveniles. In this period a total of 60 persons was convicted, out of these 15 juveniles. The court decided on inflicting a custodial sentence in 11 cases and in 45 cases the sentence was suspended. Pecuniary punishment was inflicted in 2 cases. The numerical data for the territory of The Slovak Republic are summarised in Tab. 1—4.

DISCUSSION

All cases where the prosecuted persons were accused and where the courts decided on culpability and penalties, involved proceedings according to § 203, section 2, of the Penal Code or, in 4 cases, according to § 203, section 3, of the Penal Code.

Table 1. Persons prosecuted for criminal offence of cruelty to animals in The Slovak Republic

	Total	1996	1997	1998	1999	2000	2001
Prosecuted persons	73	2	11	19	16	8	17
Juveniles from these	22	0	6	6	4	1	5

Table 2. Persons accused of criminal offence of cruelty to animals in The Slovak Republic

	Total	1996	1997	1998	1999	2000	2001
Prosecuted persons	71	2	10	18	16	8	17
Juveniles from these	21	0	6	5	4	1	5

Table 3. Persons convicted of a criminal offence of cruelty to animals in The Slovak Republic

	Total	1996	1997	1998	1999	2000	2001
Prosecuted persons	60	1	7	18	12	8	14
Juveniles from these	12	0	0	5	2	1	4

Table 4. Cases of punishment inflicted for the criminal offence of cruelty to animals in The Slovak Republic

	Total	1996	1997	1998	1999	2000	2001
Imprisonment without suspension	11	1	3	–	4	–	3
Imprisonment suspended	45	–	4	16	8	7	10
Fine	2	–	–	–	–	1	1
Stifling of prosecution	2	–	–	2	–	–	–
Total	60	1	7	18	12	8	14

The most frequent victims of violent actions were dogs, while cats and bulls were involved in 2 cases, and a heifer and horse in one case each. It was always physical violence that involved repeated attacks. The attackers used sticks, axes, rakes, a matchete, ropes, and other devices. The consequences of their actions were the same in all cases, the deliberate, violent, unauthorised killing of an animal. The violators who attacked large farm animals (bull, heifer, horse), but even those who killed dogs, stated that they were motivated by existential problems associated with unemployment.

The analyses indicated that violent actions directed against animals were taken mostly by foreign persons (not the breeders). Although cruelty to animals is not a problem involving only certain group of people, the knowledge obtained may contribute to some general conclusions:

1. With regard to the age of offenders prosecuted for cruelty to animals, a predominance of men of a young and productive age (more than one third of offenders were juveniles) was observed. In only one case the outrage was committed by three young women together with one juvenile accomplice.

2. The education level of the offenders was in general very low. They were all of them persons with basic or apprentice education. This low level was associated with the lower social rank of wrongdoers, unemployment, and a bad economic situation.

3. The knowledge obtained also indicated that in the majority of cases the incriminated action of violence for which the respective persons were prosecuted was not their only excess. It was revealed that the wrongdoers committed the violence in concurrence with other penal offences either of a violent or economic character (for example theft, burglary, and others).

4. It is noteworthy that except for individual cases all offences relating to cruelty to animals involved accomplices.

5. The personality of these offenders can be frequently characterised by a low intellectual level, emotional shallowness, and an absence of moral restraints and their actions by high degree of brutality even cynicism.

The cases recorded do not correspond to real situation because this is an area where a high potential persists and the means of penal law available punish only the most serious forms of cruelty that result in the death of animals.

Although the public seems to be rather sensitive to manifestations of cruelty to animals, the sensitivity involves rather moral condemnation than help in uncovering the cases of violence towards animals.

Undoubtedly, the criminal prosecution and conviction of the violator is not only the most effective mechanism for stopping the violence from a short-time point of view but it affects also the future behaviour of the violator.

Neither the criminal prosecution itself nor the threat of punishment can in itself be a sufficient tool for the elimination of cruelty to animals. It appears inevitable to ensure the collaboration of institutions for the protection of animals and veterinary care bodies with those responsible for protection of justice with regard to both uncovering cases of cruelty to animals and prosecuting the violators by means of various forms of justice (3).

Shortly after the Act No. 557/1991 of the Penal Code came into force, supplementing the Penal Code by § 203 Cruelty to animals, numerous organizations and groups focusing on protection of animals, but also other citizens, raised objections against section 1 (see CONCLUSION), particularly its link-up with preceding sanctions for an offence or similar criminal acts.

Judicial practice confirmed these well-founded objections.

During the entire period of observation, according to available statistical information, no offender was found guilty of cruelty to animals according to § 203, section 1, of the Penal Code.

It appears necessary to initiate an amendment of this provision, particularly its first section, which has not yet been implemented in practice.

It is more than probable that the re-codification of the Penal Code that is presently in preparation will contain § 203 Cruelty to animals amended in accordance with a need to eliminate cruelty to animals.

CONCLUSION

On the basis of the analyses of court decisions regarding the guilt and punishment of subjects charged with cruelty to animals according to § 203 of the Penal Code, this study concludes that many legal proceedings that could be described from a moral point of view as cruelty to animals of considerable violence and extent remain unpunished because of the existing legal regulations, particularly those included in section 1, § 203 of the Penal Code. We quote: *"Whoever abuses an animal although he was punished in the past year for a similar offence or was convicted for such an offence in the past two years will be condemned to imprisonment for one year, punished by an injunction against his activities or penalized"* which presumes that only an action preceded by sanctioning in the past for similar offence, having the attributes of violation or criminal offence, could be considered a criminal offence.

The results of our study confirm us in a position that corresponds with that of specialists in the property penal law that from the point of view of *de lege ferenda* there is a presumption for an amendment of the § 203 of the Penal Code.

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THE POSSIBILITY OF ENDOPARASITOSIS DAMPING BY ECOLOGICALLY CONSIDERED METHODS IN HORSES

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ABSTRACT

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

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

INTRODUCTION

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

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

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

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

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

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

MATERIALS AND METHODS

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

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

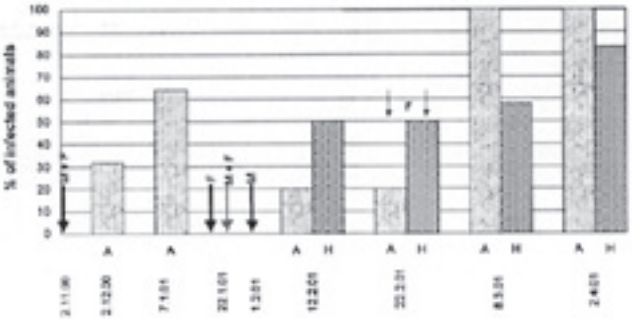

Fig. 1. A proportion of mares and foals with occurrence of Strongylidae and Trichonomatidae eggs

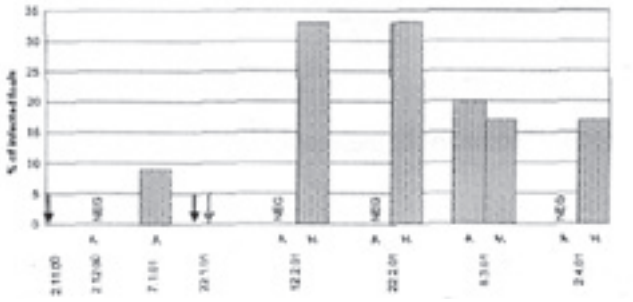

Fig. 2. A proportion of foals with occurrence of *Parascaris equorum* eggs

Dates of investigation and treatment

— allopathic treatment

— homeopathic treatment

— invetigation in foals only

F — foals; M —mares; A — allopathic; H — homeopathic

Dates of investigation and treatment

— allopathic treatment

— homeopathic treatment

NEG — negative finding; A — allopathic; H — homeopathic

group was dewormed with the preparation, Ivomec, on the day when the homeopathic treatment in the trial group ended.

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

RESULTS AND DISCUSSION

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

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

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

CONCLUSION

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

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