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CULTURAL AND BIOCHEMICAL PROPERTIES OF *ESCHERICHIA COLI*, *PROTEUS MIRABILIS* AND *KLEBSIELLA PNEUMONIAE* STRAINS FROM URINARY INFECTIONS

Maria Magdalena COSTÎNAŞ*, Svetlana ALEXA**, Rahela CARPA*

Abstract. Urinary tract infections are the most common bacterial diseases in the world population and account for the majority of the workload in the clinical microbiology laboratory. Urinary tract infections may give uncharacteristic symptoms or they could be asymptomatic or dominated by the symptoms of kidney abscess and urosepsis with associated kidney failure that may even lead to death. An accurate diagnosis and early treatment are crucial also due to a risk of long-term consequences, including chronic kidney disease. *Escherichia coli* is the most common causative pathogen for urinary tract infections. Urine test and urine culture are standard methods in the diagnostics of urinary tract infections. Urine culture remains the gold standard of urinary tract infections diagnostics, it is time-consuming and expensive. Urinary tract infections are most commonly treated using antibiotics. The aim of this paper is to highlight the current evidence of urine culture on different culture media and biochemical tests for determined urinary tract infections.

Key words: urinary tract infections, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*.

Introduction

Urinary tract infections are the most common bacterial diseases in the world population (Nzalie et al., 2016). According to an expanded surveillance program across Europe, urinary tract infections have been found to be the third most common type of infection after pneumonia and respiratory tract infections (Mladenović et al., 2015).

Urinary tract infection is a microbial inflammation that is manifested in the upper urinary tract by pyelonephritis - kidney infection, while in the lower tract is manifested by cystitis - bladder infection (Wani et al., 2016). These infections affect both sexes but are more common in women (Anjum et al., 2016). Localised are urinary tract infections of the upper tract (acute and chronic pyelonephritis, renal abscesses) and lower tract infections (cystitis, urethritis, prostatitis).

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By degree of severity there are uncomplicated infections in the upper and lower tracts such as acquired infections and infections with upper and lower tract complications such as nosocomial ones (Buiuc and Neguţ, 2009).

Urinary tract infections are manifested by pain during micturition, back pain, unpleasant, repellent urination. They are caused by different pathogens but the most common are bacteria, being responsible for about 95% of cases (Flower et al., 2016). The Enterobacteriaceae family is an important cause of urinary infections, especially *Escherichia coli*, accounting for 70-80% of the cases. Other bacteria involved in these infections are *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus* spp and *Enterococcus* spp (Anjum et al., 2016; Shatla et al., 2016).

Escherichia coli. It is a Gram negative bacil about 0.5 μm in diameter and with a length between 1 and 3 μm. They are usually mobile in liquids, indicating the presence of scarlet fever. It presents a series of biochemical features, including nitrate reduction to nitrite, and, when it develops, it ferments glucose or other carbohydrates, producing gas (H_2 or CO_2) (Nestorescu, 1965).

In the clinical laboratory, to distinguish them from other bacteria, biochemical tests such as the indole test, at which *E. coli* is positive, are used. The other tests are oxidase, urease, the use of citrate as the sole source of carbon and the test for detection of hydrogen sulphide (H_2S) production, in which most strains are negative. A characteristic that differentiates *E. coli* from other bacteria is that *E. coli* is lactose-positive on lactose-rich culture media (Paiva de Sousa, 2006).

Proteus mirabilis. It is a Gram negative, mobile molasses that degrades urea with ammonia and CO_2 formation. The bacteria is usually found in the animal and human intestines, but also in polluted waters and soils (Duca et al., 1979). Species of the genus *Proteus* are usually infectious nosocomial agents that cause not only urinary infections but also respiratory tract infections in the eyes, nose, skin, etc. Such infections involving *Proteus mirabilis* are difficult to treat, being persistent, and can lead to the formation of kidney stones (Duca, 1974; Coker et al., 2000).

In the liquid medium, *Proteus mirabilis* is a small bacillus (1.5-2 μm) showing peritrich flares. If it is transferred from the liquid medium to the solid environment, it undergoes morphological and physiological transformations, being capable of multicellular invasion (Jansen, 2003).

Klebsiella pneumoniae. This bacteria is Gram-negative, aerobic, and is a common cause of a wide range of infections in humans. It is one of the most common enteric bacteria responsible for up to 10% of all nosocomial infections and is involved in pneumonia and urinary tract infections that can cause morbidity (Khamesipour and Tajbakhsh, 2016).

The *Klebsiella* genus is a group of Enterobacteriaceae immobile that ferments glucose by producing large amounts of gas. On simple agar media

Klebsiella develops large, round, bulky, 2-3 mm thick colonies with mucoid appearance, and on low selective environments the colonies are lactose-positive, preserving the mucoid character. *Klebsiella pneumoniae* does not produce H_2S in the TSI medium and rapidly ferments lactose or sucrose (Bathia et al., 2016; Kontopidou et al., 2014).

Urinary tract infections are treated with antibiotics. The rate of medical prescriptions is influenced by the severity of the symptoms and the age of the the patients (Bleidorn et al., 2016). The treatment of urinary infections is differentiated according to the etiological agent and the type of urinary infection and must be strictly individualized in relation to the contributing factors involved, the predominant location of the infection, the antibiotic sensitivity of the bacteria, the urinary pH (Căruntu and Căruntu, 1979). Initially, a sulphamide (Sulfametoxydiazinum, Sulphafurazole, Sulfaphenazol) or co-trimoxazole (Tagremin - Sulfamethoxazole, Biseptol – Trimethoprim + sulfamethoxazole) is given. Most Gram negative bacilli are sensitive to co-trimoxazole, resulting in a healing ratio of 85% to 92% (Voiculescu, 1989). Among the most commonly used antibiotics are ampicillin, kanamycin, carbenicillin, colistin, cephalothin, gentamicin. Of broad-spectrum antibiotics, tetracyclines are most appropriate. Chloramphenicol is not indicated because it is excreted in the urine as an inactive form. *Chlamydia* is indicated for doxycycline (Campos-Franco et al., 2017). Nosocomial urinary infections in the hospital are caused by antibiotic-resistant bacteria. In such a case, antibiotics are indicated, as carbenicillin, tricarcillin, aminoglycosides, ureidopenicillins, cephalosporins, 3rd generation quinolones (Voiculescu, 1989). Quinolones are the most used antibiotics to treat infections caused by *Escherichia coli* and *Klebsiella pneumoniae* in humans and animals (Daoud et al., 2014). The length of treatment in acute infections is 10-14 days. The therapeutic failures are treated with repeated treatments and the change of antibiotic depending on the sensitivity of the germ (Voiculescu, 1989).

Materials and methods

Specimen collection, transportation and handling of urine. Regardless of the sampling mode, urine specimens should be microbiologically examined within 2 hours (Alteraş et al., 1962). The urinal summary exam is a screening test useful for highlighting certain conditions, but is not sufficient to establish a diagnosis. It is made from the first urine harvested in the morning and includes: the macroscopic examination (volume, appearance, color, smell), physical examination (density and pH), chemical examination (nitrite, protein, glucose, ketone, pus, bilirubin, urobilinogen) microscopic exam (cells, cylinders, bacterial flora, crystals) (Dobreanu, 2002).

Uroculture and biochemical tests. Different types of nutrient media are used for uroculture: basic environments and enriched environments for general use. Sowing samples is done on blood agar, CLED-Cystine-Lactose-Deficient

Electrolyte or on Selective Media for Gram-negative Bacteria (Buiuc and Neguţ, 2009). Among the most commonly used selective media are: M.I.U. (Mobility Indol Urea), T.S.I. (Triple Sugar-Iron-Agar) and Simmons (citrate) (Atlas, 2010).

Susceptibility of bacteria to antibiotics. The antibiotic test is performed for any isolated germ. This testing of the sensitivity of a bacterial strain to antimicrobial agents is one of the most frequent examinations. For the clinical laboratory, a diffusimetric method is recommended, which is easier to perform, more economical, and, if strictly complies with all working conditions, it can provide a satisfactory reproducibility. The Kirby-Bauer method is applied in most countries and is relatively (Buiuc and Neguţ, 2009). By placing antibiotic disks on the surface of a solid medium inocuated with a bacterial culture, the active antimicrobial substance will diffuse in the medium with a constant decrease in the concentration gradient from the edge of the disc to the periphery (Carpa et al., 2014). The culture medium is Mueller-Hinton (MH) agar which allows the optimal development of a large variety of germs and contains no inhibitors of the action of antimicrobial substances (Lazăr et al., 2004).

Expression of the results in the bulletin is done by direct transcription of the inhibition zone diameter into categories of susceptible, resistant or intermediate strains, by marking the diameter of the inhibition zone (Buiuc and Neguţ, 2009).

Results and Discussions

Assessment of cultural properties

The most common bacteria that spreads urinal infection is *Escherichia coli*, followed by bacteria as *Proteus mirabilis* and *Klebsiella pneumoniae*.

The growing of *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* isolated from urine on blood-agar culture media.

Colonies of *Escherichia coli* have the following features: colonies of type S (rounds), polish, grey color (Figure 1a).

Proteus mirabilis is an invading species, spreading at all culture media surface. Colonies have grey color, type S (Figure 1b).

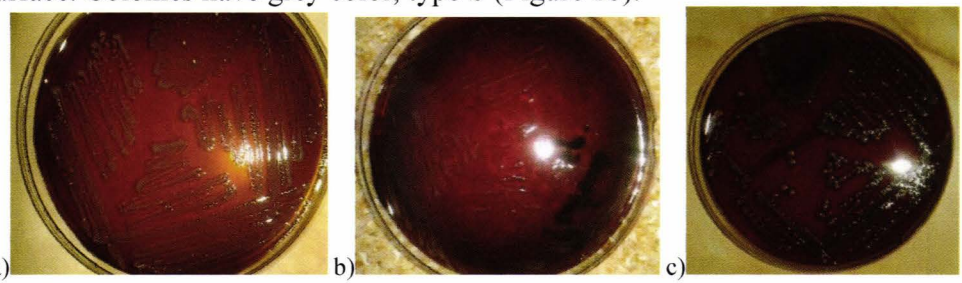

Fig. 1. Colonies of a) *Escherichia coli,* b) *Proteus mirabilis* and c) *Klebsiella pneumoniae* on blood-agar culture media

Klebsiella pneumoniae on agar-blood have the following features: the spreading is abundant, colonies are type M (snotty) and the color is grey. The aspect is highlighted in Figure 1c.

The growing of *Escherichia coli, Proteus mirabillis* and *Klebsiella pneumoniae* isolated from urine on CLED culture media.

On CLED (Cystine-Lactose-Defficient Electrolyte) culture media, *Escherichia coli* bacteria has the following macroscopic features: spread is abundant, diameter ranges between 2.0 and 3.0 mm, colonies are type S (rounds) and color is yellow opal. The media culture is changing his color passing from green color to yellow, because the bacteria is lactose-positive (Figure 2a).

Fig. 2. Colonies of a) *Escherichia coli,* b) *Proteus mirabilis* and c) *Klebsiella pneumoniae* on CLED-agar

Colonies of bacteria *Proteus mirabilis* are transparent, the color is green like the CLED culture media, type S (rounds). Diameter is between 2 and 3 mm, the spreading is abundant. Bacteria is lactose-negative. This aspect is observed in figure 2b.

The features of bacteria *Klebsiella pneumoniae* on CLED culture media are: type M colonies (snotty), diameter between 3.0 and 4.0 mm, abundant spreading and yellow colonies. The bacteria is lactose-positive and it can be observed in figure 2c.

The spreading of *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* isolated from urine on M.I.U. (Mobility Indole Urea)

The features of *Escherichia coli* are: negative urea, positive indole, it is movable and the culture media doesn't change his color (Figure 3a).

Colonies of *Proteus mirabilis* bacteria have the following features: urea positive, indole negative, the color of the culture media is changing to pink and this aspect is highlighted in Figure 3b.

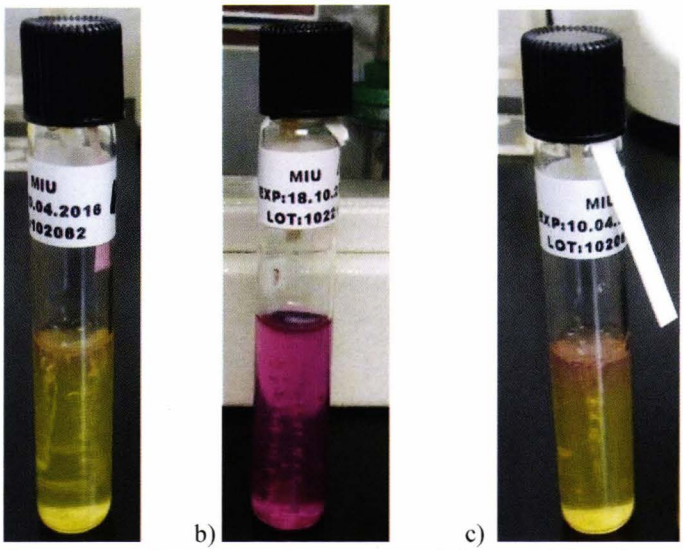

Fig. 3. Colonies of a) *Escherichia coli;* b) *Proteus mirabilis*; c) *Klebsiella pneumoniae* on M.I.U. culture media

Klebsiella pneumoniae has the following features: positive urea, negative indole and the M.I.U media culture is changing his color to pink (Figure 3c).

The spreading of *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* isolated from urine on T.S.I Agar (Triple Sugar-Iron-Agar).

At *Escherichia coli* bacteria the color of the media culture is yellow, because is lactose-positive, and the bacteria is fermenting glucose without producing H_2S or other gas. The growing is abundant and can be observed in Figure 4a.

The growing of *Proteus mirabilis* is abundant, bacteria is lactose-negative. This bacteria is fermenting glucose and produces H_2S without other gas. In the presence of H_2S the color of the culture media is changing from red to black, this aspect is observed in Figure 4b.

Klebsiella pneumoniae has the following features on TSI media culture: the spreading is abundant, bacteria is lactose-positive, it ferments glucose and sucrose, doesn't produce H_2S only gas. The color of culture media is changing from red to yellow and this aspect can be observed in Figure 4c.

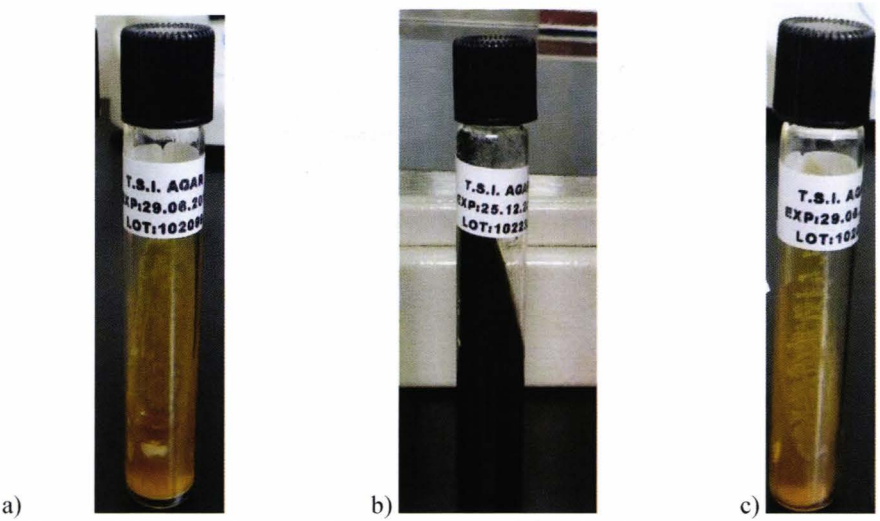

Fig. 4. a) *Escherichia coli*; b) *Proteus mirabilis*; c) *Klebsiella pneumoniae* on TSI culture media

The spreading of *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* isolated from urine on Simmons culture media (citrate culture media).

Escherichia coli bacteria don't consume citrate as a source of energy. The color of the culture media remains unchanged, green (Figure 5a).

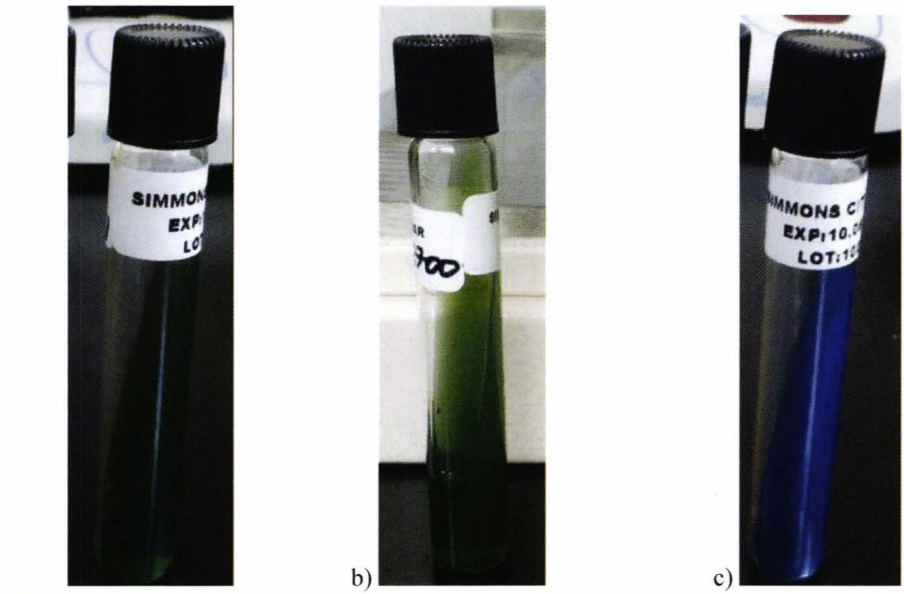

Fig. 5. a) *Escherichia coli*; b) *Proteus mirabilis*; c) *Klebsiella pneumoniae* on Simmons culture media

Proteus mirabilis bacteria consume citrate as the only source of energy and it changes the color from green to blue, aspect that can be observed in Figure 5b.

Klebsiella pneumoniae, like the *Proteus mirabilis*, it changing her color in blue (Figure 5c).

Antibiograms

After the result of pure analysis, the next phase is to analyze the antibiograms. There were analyzed 3 antibiograms for each bacteria. The antibiotics that have been used for each antibiogram and the degree of sensitivity - resistance can be observed in table 3.

Table 3. Antibiotics uses in bacterials susceptibility tests

Symbol	Name	Diameter of inhibition zone (mm)	
		Sensitivity (≥)	Resistant (≤)
AM – 10	Ampicillin	14	14
SAM – 10	Ampicillin - sulbactam	14	14
AMC – 10	Amoxicillin - clavulanic	17	17
CFM – 5	Cefixime	17	17
CAZ – 10	Ceftazidime	22	19
CXM – 30	Cefuroxime	18	18
CN – 10	Gentamicin	17	14
F/NIT – 100	Nitrofurantoin	11	11
COT/SXT – 1.25 – 23.75	Trimethoprim - sulfamethoxazol	16	13
CIP	Ciprofloxacin	22	19
NA	Nalidixic acid	16	14
CRO	Ceftriaxone	23	20

For *Escherichia coli* the following antibiotics were used: gentamicin (CN – 10 μg) ceftriaxone (CRO 30 μg), ceftazidime (CAZ – 10 μg), cefuroxime (CXM – 30 μg), trimethoprim – sulfamethoxazol (COT/SXT – 1.25 – 23.75 μg), nitrofurantoin (F/NIT – 100 μg), ampicillin – sulbactam (SAM – 10 μg), amoxicillin – clavulanic (AMC – 10 μg) and cefixime (CFM- 5 μg) (Figure 6).

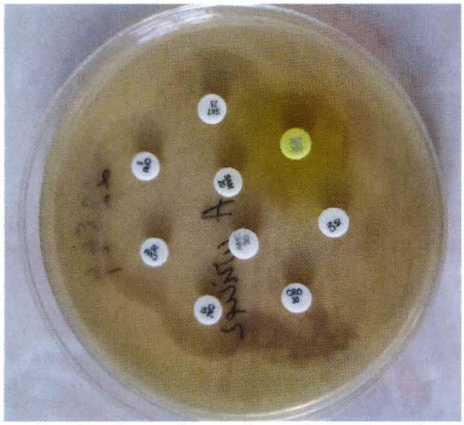

Fig. 6. Antibiogram for *Escherichia coli* on Mueller-Hinton culture medium

For *Proteus mirabilis* following antibiotics were used the: nalidixic acid (NA 30 µg), gentamicin (CN – 10 µg), ceftriaxone (CRO 30 µg), ceftazidime (CAZ – 10 µg), cefuroxime (CXM – 30 µg), trimethoprim – sulfamethoxazol (COT/SXT – 1.25 – 23.75 µg), ciprofloxacin (CIP 5 µg), nitrofurantoin (F/NIT – 100 µg), ampicillin (AM – 10 µg), ampicillin– sulbactam (SAM – 10 µg), amoxicillin – clavulanic (AMC – 10 µg) (Figure 7).

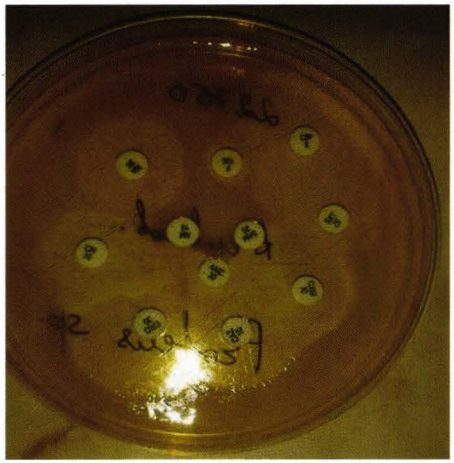

Fig. 7. Antibiogram for *Proteus mirabilis* on Mueller-Hinton culture medium

For *Klebsiella pneumoniae* there were used the following antibiotics: acid nalidixic (NA 30 µg), gentamicin (CN – 10 µg), ceftriaxone (CRO 30 µg), ceftazidime (CAZ – 10 µg), cefuroxime (CXM – 30 µg), trimethoprim – sulfamethoxazol (COT/SXT – 1.25 – 23.75 µg), ciprofloxacin (CIP 5 µg), ampicillin (AM – 10 µg), ampicillin – sulbactam (SAM – 10 µg) and amoxicillin-clavulanic (AMC – 10 µg) (Figure 8).

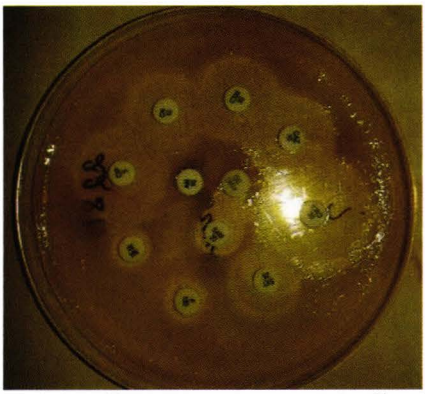

Fig. 8. Antibiogram for *Klebsiella pneumoniae* on Mueller-Hinton culture medium

The results of the antibiograms are displayed in tabels 4, 5 and 6, and based on the diameters of inhibition, the sensitivity of the bacteria was assessed. The diameter of inhibition reveals the sensitivity of *Escherichia coli* in contact with a specific antibiotic. It can be observed that the Ceftriaxone (30 mm) caused the biggest inhibition.

Table 4. Antibiogram interpretation of bacteria *Escherichia coli* from uroculture

Name antibiotic	Symbol	Diameter of inhibition	Sensitivity (≥)	Resistant (≤)
Gentamicin	CN – 10 µg	20 mm	√	
Ceftriaxone	CRO 30 µg	30 mm	√	
Ceftazidime	CAZ – 10 µg	22 mm	√	
Cefuroxime	CXM – 30 µg	24 mm	√	
Trimethoprim - sulfamethoxazol	COT/SXT – 1,25 – 23,75 µg	26 mm	√	
Nitrofurantoin	F/NIT – 100 µg	22 mm	√	
Ampicillin - sulbactam	SAM – 10 µg	18 mm	√	
Amoxicillin - clavulanic	AMC – 10 µg	18 mm	√	
Cefixime	CFM- 5 µg	24 mm	√	

Based on the diameter of inhibition was also established the sensitivity of *Proteus mirabilis* in contact with an antibiotic, the reaction can be observed in table 5. It can be observed that Ciprofloxacin (36 mm) and Ceftriaxone (32 mm) cause the biggest inhibition.

Table 5. Antibiogram interpretation of bacteria *Proteus mirabilis* from uroculture

Name of antibiotic	Symbol	Diameter of inhibition	Sensitivity (≥)	Resistant (≤)
Nalidixic acid	NA 30 μg	23 mm	√	
Gentamicin	CN – 10 μg	20 mm	√	
Ceftriaxone	CRO 30 μg	32 mm	√	
Ceftazidime	CAZ – 10 μg	24 mm	√	
Cefuroxime	CXM – 30 μg	21 mm	√	
Trimethoprim - sulfmethoxazol	COT/SXT – 1.25 – 23.75 μg	14 mm		√
Ciprofloxacin	CIP 5 μg	36 mm	√	
Nitrofurantoin	F/NIT – 100 μg	8 mm		√
Ampicillin	AM – 10 μg	6 mm		√
Ampicillin - sulbactam	SAM – 10 μg	6 mm		√
Amoxicillin - clavulanic	AMC – 10 μg	6 mm		√

In table 6 can be noticed that the bacteria is resistant to only 4 antibiotics from 10 and is sensitive at: gentamicin, ceftriaxone, ceftazidime, cefuroxime, ciproflaxin and trimethoprim – sulfmethoxazol.

Table 6. Antibiogram interpretation of bacteria *Klebsiella pneumoniae* from uroculture

Name of antibiotic	Symbol	Diameter of colony	Sensitivity (≥)	Resistant (≤)
Nalidixic acid	NA 30 μg	12 mm		√
Gentamicin	CN – 10 μg	19 mm	√	
Ceftriaxone	CRO 30 μg	27 mm	√	
Ceftazidime	CAZ – 10 μg	21 mm	√	
Cefuroxime	CXM – 30 μg	20 mm	√	
Trimethoprim - sulfamethoxazol	COT/SXT – 1.25 – 23.75 μg	17 mm	√	
Ciprofloxacin	CIP 5 μg	26 mm	√	
Ampicillin	AM – 10 μg	6 mm		√
Ampicillin - sulbactam	SAM – 10 μg	12 mm		√
Amoxicillin - clavulanic	AMC – 10 μg	6 mm		√

Conclusions

For urinary tract infections there are some types of analyses, as uroculture, biochemical testing of isolated strains and antimicrobial activity. After the sampling of the product (urine), it was analyzed on different culture media as Agar-Blood and CLED culture media. Biochemical testing of the three strains involved in urinary infections was performed on selective culture media like MIU, TSI and Simmons.

Species as *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* have developed colonies on the culture media with different species specific features (different shapes, different colors and different macroscopic traits).

After obtaining the pure cultures, the antibiograms for assessing their sensitivity were performed.

- *Escherichia coli* isolated from uroculture exhibits sensitivity at all the studied antibiotics.
- *Proteus mirabilis* isolated from uroculture was resistant to different types of ampicilline but also to Trimethoprim – sulphmethoxazole and Nitrofurantoin.
- *Klebsiella pneumoniae* isolated from uroculture was resistant to nalidixic acid and to different types of ampicilline.

Rezumat. Infecţiile tractului urinar sunt cele mai comune boli bacteriene în cadrul întregii populaţii a globului şi acestora li se alocă majoritatea volumului de muncă în laboratorul clinic de microbiologie. Infecţiile tractului urinar pot cauza simptome nespecifice sau pot fi asimptomatice sau dominate de simptomele abcesului renal şi ale urosepticemiei cu insuficienţă renală asociată care poate duce chiar şi la deces. Un diagnostic corect şi tratarea timpurie sunt cruciale, de asemenea, datorită riscului privind consecinţe pe termen lung, incluzând îmbolnăvirea cronică a rinichilor. *Escherichia coli* este cel mai comun patogen responsabil pentru infecţiile tractului urinar. Testul de urină şi urocultura sunt metode standard în diagnosticarea infecţiilor tractului urinar. Urocultura rămâne standardul esenţial pentru diagnosticarea infecţiilor tractului urinar, deşi necesită timp şi este costisitor. Infecţiile tractului urinar sunt de cele mai multe ori tratate cu antibiotice. Scopul acestei lucrări este să prezinte date privind urocultura pe diferite medii precum şi testele biochimice pentru anumite infecţii ale tractului urinar.

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THE ACTION OF ENVIRONMENTAL FACTORS ON THE CONDITION OF THE FACIAL SKIN

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Abstract. Facial skin is one of the zones most exposed to different environmental factors which can interfere in the metabolic processes localised at this level or can affect its morphology of anatomy. These environmental factors influence one of the most important processes, namely skin ageing. In this paper the acute and chronic effects of face skin exposure to several environmental factors are described. The factors having the greatest influence on the physiology and anatomy of the face skin are solar radiation and low temperatures. Some prevention measures against the impairments specific to excessive exposure to sun of low temperatures are also described. Excessive exposure to sun causes several impairments at skin level, but a regular exposure is needed for the good condition of the organism, including synthesis of vitamin D. Regarding low temperatures, the limitation of exposure time, protection of exposed areas by suitable wear, avoiding dehydration, using creams and diverse unguents for preventing desquamation and humidity evaporation at face skin level are indicated.

Key words: skin, cold temperature, photodamage, protection, prevention.

Introduction

The skin is a complex organ with several vital functions. The skin, especially on the face, beside protection and isolation, has an important role in defining one's physiognomy and looks through anatomical variations from one individual to other. The skin on the face represent one of the most exposed areas of this organ to different environmental factors. These extrinsic factors can interfere with metabolic processes at this level or can affect the anatomy and the morphology of the skin (D'Orazio et al., 2013; Flament et al., 2014; Rittié and Fisher, 2015).

The most important process that is influenced by the action of the environmental factors is the natural and inevitable process of skin ageing. Besides the intrinsic factors (genetic predispositions, skin type, metabolic processes, hormones, etc.) that produce ageing, several ennvironmental factors such as UV light, cold temperatures, smoking, toxins and pollution can cause or can aggravate the ageing of the skin (Farage et al., 2008; Flament et al., 2013; Ganceviciene et al., 2012).

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In this paper we address the effect of solar radiation and cold temperature on the physiology and anatomy of the facial skin complexion.

1. The effects of cold exposure to the skin

The complexion is especially exposed to the effect of cold temperature, due to the difficulty of covering all areas of the face. Compared to the lesions and cold exposure injuries to the extremities, the injuries localised on facial skin (especially frostbites) are less severe and less frequent, due to the complex structure of the skin in this area (Golant et al., 2008).

Exposure to low temperature can cause acute symptoms that may disappear with the rewarming of the affected area or chronic symptoms that may be aggravated with the rewarming of the injured skin. These injuries can range from simple lesions to serious injuries or even pathological conditions (Kellar et al., 2016; Snowise and Dexter, 2004).

1.1. Acute symptoms

The first symptoms that appear at the exposure of the facial skin to cold temperature are: vasoconstriction, redness, numbness, pallor, inflammation, dehydration and desquamation of the skin. These symptoms disappear with the rewarming of the skin (Lehmuskallio et al., 2002).

1.2. Severe cold injuries

On a long term, prolonged cold exposure causes the intensification of skin descuamation, reduction of sebum secretion (which acts as a protective layer and traps moisture next to the skin), aggravation of vasocontriction which may lead to ischemic areas of the facial skin (Kellar et al., 2016). Also, it leads to changes in colour and texture of the skin, it appearing as opaque, dull, pall and with hint of yellow (Lewis, 1941). The most common cold injuries are chilblains, frostnip and frostbite (Fig. 1) (Steegmann, 1970).

Chronic injuries or severe cold injuries that can appear after cold exposure can be classified in non-freezing and freezing injuries. Non-freezing and freezing cold exposure injuries occur especially at the extremities, but they can also occur on facial skin. These injuries can range in severity from mild, superficial injuries with few or no clinical sequelae, to severe, deep injuries with serious long-term problems (Golant et al., 2008; Lehmuskallio et al., 2002).

Freezing cold injuries (FCIs) are defined as the damage sustained by tissues while subject skin temperature drops to temperatures below their freezing point at approximately −0.55°C. Non-freezing injuries (NFCIs) occur when tissue fluids are continuously exposed to low temperatures between 0 and 15°C for several hours or days (Heil et al., 2016; Imray et al., 2009).

Frostnip is a mild form of freezing injuries and it is reversible if further exposure is prevented. Affects only the superficial layers of the skin, without affecting the dermis or deeper tissues. It presents itself with pallor, numbness, pain and skin blanching (Golant et al., 2008; Hota and Singh, 2013; Jurkovich, 2007).

Frostbite represents a severe type of cold exposure injury. It occurs in below-zero temperatures, when the skin is exposed for a prolonged time. This type of injury develops due to the direct formation of ice crystal at cellular level, cellular dehydration and microvascular occlusion (Jurkovich, 2007). This type of condition can be classified in four categories after the degree of tissue damage: first degree frostbite (numb central white plaque surrounded by erythema, without blisters and necrosis), second degree frostbite (clear blisters surrounded by erythema and edema, which appear during the first 24 hours after the exposure), third degree frostbite (formation of hemoragic blisters, thromboses and black eschars) and four degree frostbite (tissue necrosis, gangrene). Initially all frostbites appear to be similar at first look and their correct evaluation can be done only after the rewarming of the affected skin area (Golant et al., 2008; Heil et al., 2016; Hota and Singh, 2013; Jurkovich, 2007; Lehmuskallio et al., 2002).

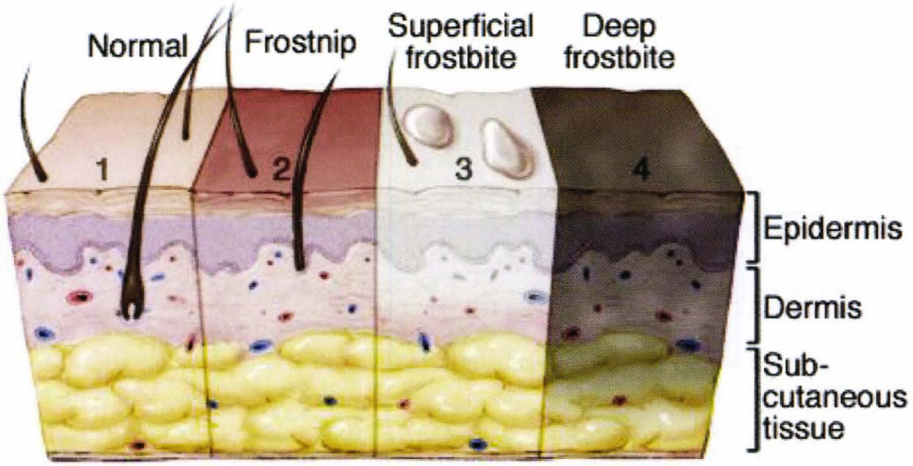

Fig. 1. Cold exposure injuries (https://www.mayoclinic.org/-/media/kcms/gbs/patient-consumer/images/2014/10/10/10/32/r7_frostbite.ashx)

Severe symptoms can appear after the rewarming of a frostbite affected skin area, such as blisters, severe pain, edema ischemia, cyanosis, gangrene or circulatory failure (Imray et al., 2009). This suggest that a frosbite injury is composed of two elements; the initial freeze injury and the injuries that occur during the rewarming of the affected area (Jurkovich, 2007).

The development of a frostbite injury is usually progressive, starting with mild symptoms continued with the development of severe symptoms and usually ending with localised or extensive necrosis of skin and underlying tissues or there may be healing with sequelae or complete heal without clinical symptoms (Golant et al., 2008; Heil et al., 2016). Minor and superficial frostbites occur in areas of the face, meanwhile deep frostbite injuries affect the extremities (Golant et al., 2008).

Both frostnip and frostbite can be recognized clinically, but many times during initial stages the severity and the extent of the injury can be overlooked or it is difficult to determine exactly the severity (Heil et al., 2016).

A type of nonfreezing injuries is chilblains, which occur in response to repeated exposure to low temperature in dry conditions. Individuals who develop this condition experience a burning sensation, pruritus, erythema and swelling of the affected area and sometimes blistering, ulceration, red purple plaques or nodules can develop, too (Golant et al., 2008; Hota and Singh, 2013; Jurkovich, 2007).

2. The clinical effects of solar radiation on skin

Sun exposure is virtually impossibile to avoid and a regular exposure is necessary for normal functioning of the organism, especially for the synthesis of vitamin D (Flament et al., 2013). However, if the exposure is prolonged and no measures of protection are taken, solar radiation, ultraviolet (UV) radiation in particular, can cause a different range of symptoms from mild to severe states (Young et al., 2017).

The main component of the solar radiation that is responsible for the structural, physiological changes of the facial skin is ultraviolet radiation, also being a carcinogen agent. UV radiation can be classified in 3 categories after wavelenght: UVC (100-290 nm, mostly blocked by the atmosphere), UVB (290-320 nm, which penetrates only the epidermis and affect predominantly keratinocytes and it is responsible for the immediate changes on the skin) and UVA (penetrates the derm and can interact with keratinocytes and dermal fibroblasts, it is responsible for chronic symptoms and signs associated with photoageing) (Fig. 2) (Berneburg et al., 2000; D'Orazio et al., 2013; Farage et al., 2008).

The different types of UV radiation, after they reached the specific layers of the skin, exert their specific effects: UVA is responsible for the generation of reactive oxygen species, meanwhile UVB is responsable for DNA damage after direct interaction with the DNA structure (Berneburg et al., 2000; Farage et al., 2008). The direct damages include the formation of pyrimidine dimers, especially thymine dimers, that result in mutations in the DNA and can affect important gene function and structure. Mutations caused by UV radiation in *p53* gene were found in the majority of actinic keratoses, squamous cell carcinomas and basal

cell carcinomas (Holick, 2010; Sklar et al., 2013). DNA damage and mutations of the mitochondrial DNA (especially large-scale deletions) can affect mitochondrial function and thus leading to further accumulation of reactive oxygen species (ROS) (Berneburg et al., 2000; Yaar and Gilchrest, 2007).

The changes of the skin due to sun exposure are the results of changes in molecular mechanism due to the formation of reactive oxygen species. These compounds can interfere with numerous cellular processes and can affect the structure of important macromolecules such as proteins, DNA, RNA, which can lead to loss or gain of activity of these molecules (Ganceviciene et al., 2012; Yaar and Gilchrest, 2007). Endogenous antioxidants, such as superoxid dismutase, catalase, GSH peroxidase, vitamin E, uric acid, and, most important, melanin have an important role in destroying these ROS (Ganceviciene et al., 2012; Pandel et al., 2013).

Along the production of ROS, UV radiation can penetrate the epidermis and dermis, can disturb the tightly regulated synthesis of enzymes, such as collagenase, gelatinase (matrix metalloproteinases), through the activation of specific transcription factors. The result of this process is the induction of these enzymes responsible for degrading collagen and elastin molecules, which are key elements of the dermal extracellular matrix (Benderburg et al., 2000; Rittié and Fisher, 2015; Yaar and Gilchrest, 2007). These disturbances affect the post-translational modification, resulting in a change in the structure of these proteins. The modification of the structure of these proteins is translated into a loss of elasticity of skin, modification of the complexion texture (solar elastosis) (Benderburg et al., 2000). Furthermore, intense UV radiation and repeated exposure can interfere with repair processes of the extracellular matrix which determines the accumulation of permanent lesions and damage (Farage et al., 2008; Rittié and Fisher, 2015; Deng et al., 2015).

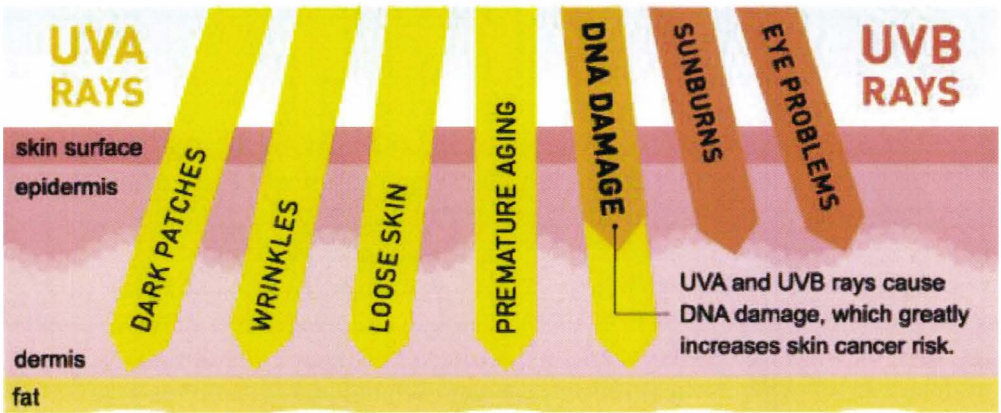

Fig. 2. Types of UV radiation and their effects on the skin (http://mdmflow.com/wp-content/uploads/2017/04/skin-cancer.png)

UVR, alongside its carcinogenic effect, can promote cancer growth and development through the inflammatory processes associated with excessive exposure (Farage et al., 2008).

Besides UV radiation, infrared (760 nm - 1 mm) accounts for approximately 40% of the solar radiation reaching the Earth. Infrared radiation (IR) can penetrate all the layers of the skin and it is perceived as heat. By over-warming the skin, IR can induce acute symptoms such as thermal pain, temporary erythema and even cardiovascular collapse and in the case of excessive exposure it may induce chronic symptoms such as squamous cell carcinoma and erythema (Sklar et al., 2013; Sarkar and Gaddameedhi, 2018).

The clinical effects of UVR on normal-appearing human skin may be acute or chronic (Farage et al., 2008).

Due to the fact that every individual is exposed to the sun during his lifetime, skin lesions and injuries are very common, but their number and severity is linked to the behavior concerning sun exposure (Young et al., 2017)

2.1. Acute symptoms

Symptoms that appear shortly after exposure to high intensity radiation are the most common and usually do not present further complications. These symptoms range from redness, rashes, erythema, pigmentation, inflammation, pain, solar urticaria, sunburn with different severity degree (Farage et al., 2008; Young et al., 2017).

Primarily, the skin responds to the UV radiation by the intensification of metabolic processes, such as the synthesis of melanin, biological pigment responsible for the absorption of the majority of the UV radiation (thus protecting skin cells from the effect of UV exposure) and by intensifying the activity and growing the number of melanocytes, resulting in the development of a tan (Berneburg et al., 2000; Gange and Parrish, 1983, Solovan and Chiticariu, 2013).

2.2. Chronic symptoms

Prolonged exposure at periods when the intensity of the UV radiation is high (at noon), can cause, beside the acute symptoms mentioned earlier, chronic symptoms and pathological states that can create, besides discomfort, serious health problems. Main chronic symptoms include photocarcinogenesis and photoageing. All symptoms are underlyingly caused by molecular or cellular effects of UV radiation such as DNA damage, the generation of ROS (singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals), disruption of normal metabolic and physiological processes such melanogenesis, apoptosis, mutation of genomic and mitochondrial DNA, expression of many genes and related proteins (Berneburg et al., 2000; Young et al., 2017).

The main effect of the UV radiation exposure is premature aging of the skin known as photoageing. It is a complex and cumulative process that is characterised by clinical, histological and biochemical changes and which is very similar and tightly connected with the natural ageing of the skin. The number of lesions, marks, injuries associated with this type of ageing of the skin is correlated with the exposure time to highly damaging UV radiations (Berneburg et al., 2000; Fisher et al., 2002).

It may be hard to differentiate the symptoms associated with normal ageing of the skin to those associated with photoageing, because all individuals present varying symptoms associated with inevitable sun exposure. Photoageing of the skin which occurs during the life of an individual can be very similar with chronological ageing, partly overlapping and superimposing with the symptoms of natural ageing (Berneburg et al., 2000; Fisher et al., 2002; Rittié and Fisher, 2015). Though, photoageing is considered only to accelerate intrinsic ageing, it is important to make a distinction between these two types of ageing (Kappes and Elsner, 2003).

Beside the face, signs of photoageing are primarily located on frequently exposed areas such as hands, forearms and neck (Rittié and Fisher, 2015).

Injuries due to solar exposure can be located in the epidermis (pigmentation, actinic keratosis) dermis (solar elastosis), sebaceous glandes, melanocytes, blood vessels (telangiectasia) (Chung et al., 2003; Jackson, 2001).

The lesions and injuries associated with photoageing can be classified into cosmetic ones, such as wrinkles, pigmentation, redness or can be pathological such as state of chronic inflammation, telangieactasia, cancers: melanoma, squamous cell carcinoma, basal cell carcinoma (Jackson, 2001, Pour et al., 2015). These are the most common signs of photoaged skin but there is a wide quantitative and qualitative interindividual variation due to difference in skin type, sun-exposure behaviour (Berneburg et al., 2000).

The main aesthetic signs associated with photoageing are hipo- and hyperpigmentation, deep wrinkles, dry epidermis, skin laxity, degradation of texture, leathery skin appearance, increased roughness, skin colour changes (sallow yellow colour), keratosis and elastosis. These signs can be differentiated from those characteristics of normal ageing such as fine wrinkles (rhytides) and lines, atrophy and thinning of the skin, general dry look of the complexion (Chung et al., 2003; Flament et al., 2013; Ganceviciene et al., 2012). Flament et al., (2013) suggested that 80% of the characteristic of aged skin are caused by UV radiation, while Farage et al., (2008) suggested that account for up to 90%.

The most important and characteristics signs of photoageing are pigmentation and development of deep wrinkles (Ganceviciene et al., 2012).

Pigmentation of the skin is characteristic to normal skin ageing, but also to ageing caused by UV radiation (Flament et al., 2014). This pigmentation occur due to a high synthesis of melanin or a growth in number of melanocytes. The

most frequent pigmentations linked to photoageing are: brown spots known as freckles (ephelides) or larger spots such as lentigiosis, solar purpura and solar keratosis (Lowe, 2006). The formation of wrinkles is associated with a higher synthesis of melanin. Solar lentigiosis (lentigine - a benign macula well delimited, surrounded by skin with normal appearance) is associated with an increased number of melanocytes formed through division after sun exposure (Farage et al., 2008; Jackson, 2001; Solovan and Chiticariu, 2013). Solar keratosis is pre-cancerous patch of thick, scaly, or crusty skin (Young et al., 2017).

Solar elastosis is one of the most prominent histological feature of photoaged skin, which is located at the junction of papillary and reticular dermis. It is the result of the low concentration and dispersion of altered collagen and elastic fibers. It is not observed in normal aged skin (Berneburg et al., 2000; Ganceviciene et al., 2012; Wulf et al., 2004).

The dermis affected by UV radiation loses its role of support for blood vessels due to the reorganization of the extracellular matrix and dispersion of collagen. This allows blood vessels to grow their diameter and thicken their walls, becoming visible on the face, resembling a network of wires, especially around the nose, chin and cheeks areas. This condition is known as telangiectasia (Farage et al., 2008; Yaar and Gilchrest, 2007).

It has been shown that individuals with lighter skin (UV sensitive) who spend a lot of time in the incidence of solar radiation, over repeated amounts of time, are more prone to morphologic anatomic and physiological changes which affect the skin and other organs (D'Orazio et al., 2013; Farage et al., 2008). Six Fitzapatrick skin phototypes (I-VI) were described and they take in account skin colour and the response to sun exposure in the form of burning or tanning. The types of skin range from very fair skin (I), more likely to sunburn and to develop cancer, to very dark skin (VI) likely to tan and rarely to burn (Sachdeva, 2009; Young et al., 2017). Skin complexion and UV sensitivity are determined by the amount and the type of melanin found in the skin (D'Orazio et al., 2013).

Due the increased level of melanin in the case of darker skinned individuals, signs of photoageing appear at much later stages than at fair-skinned people and the incidence of skin cancer is much lower (Bernburg et al., 2000; Bradford, 2009).

It has also been shown that signs of early ageing of the skin can appear already in adolescence and signs appear much earlier in life at individuals who actively seek to expose themselves to the sun compared with other individuals (Farage et al., 2008; Fisher et al., 2002; Jackson, 2001).

Besides photoageing, photodamage of the skin can be present in the form of photocarcinogenesis. The main types of cancer associated with UV radiation exposure are melanoma, squamous cell carcinoma and basal cell carcinoma. It

is believed that UV is the cause for nearly 65% of melanoma and 90% of non-melanoma skin cancers (D'Orazio et al., 2013).

The main type of neoplasm which occurs on the skin is melanoma. It is the most deadly, metastasis prone and aggressive form of cancer among those that affect the skin. It presents itself with symmetrical hyperpigmentation with uneven colour and borders and the capacity of changing shape and size (Fig. 3) (Dennis et al., 2008; Solovan and Chiticariu, 2013). It has been shown that repeated severe sunburns can be a factor of risk for the development of melanoma and due to modern trends of being tanned, the rate of skin cancers have grown significantly in the last 50 years (Dennis et al., 2008; D'Orazio et al., 2013).

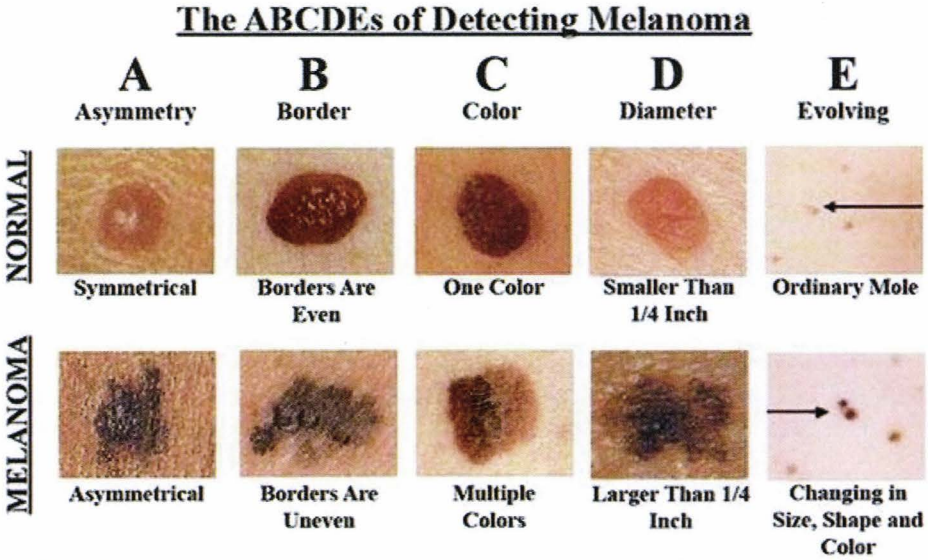

Fig. 3. Characteristics of melanoma compared to normal skin pigmentations (http://www.skincancer.org/Media/Default/Page/skin-cancer-information/Melanoma/d.jpg)

Basal cell carcinomas and squamous cell carcinomas, known as non-melanoma skin cancers or keratinocyte cancers are the most common forms of skin cancer in the world (Young et al., 2017). The name keratinocyte cancer is given by the fact that both types of neoplasm are derived from epidermal keratinocytes. Compared to melanoma, they do not metastasize (remain in the primary site) and are much easier to treat, having a better long-term prognosis (D'Orazio et al., 2013; Madan et al., 2010; Tarbuk et al., 2016). Basal cell carcinoma occurs on the head and neck and it manifests with the development of small, translucent tumours in the basal layer of the epidermis, with telangiectatic edges (Dubas and Ingraffea, 2013; Madan et al., 2010). Four subtypes of basal cell carcinoma exist: superficial, nodular ulcerative,

pigmented and morpheaform. Squamous-cell carcinoma develop from precursor lesions, especially from actinic keratosis (Madan et al., 2010).

3. Prevention and protection

Prevention of the lesions, injuries and modifications that can occur due to exposure to environmental factors is important for maintaining a healthy skin and organism.

In case of sun exposure, it is recommended to avoid exposure during high intensity UV radiations and limit the exposure time. Furthermore, the use of s sun protective creams, oils, lotions which protect against UVA and UVB has been shown to lower the number and the severity of lesions associated with sun exposure, having protective effect against photocarcinogenesis and photoageing (Pour et al., 2015; Snowise and Dexter, 2004; Young et al., 2017). The ability of blocking the UV (UVA and UVB) radiation is given by the chemical or physical compounds found in these products. The chemical absorbers convert the absorbed high-intensity UV radiation to heat. Sunscreen materials are divided into organic sunscreen and inorganic sunscreen. Organic sunscreen materials absorb UV radiations, while inorganic sunscreen materials are able to reflect UV radiations (Heo et al., 2018; Sambandan and Ratner, 2011) The inorganic sunscreens contain photostable compounds, such as zinc oxide and titanium dioxide that have the ability to reflect and scatter UV light (Antoniou et al., 2010; Yaar and Gilchrest, 2007). Other, like polysaccharide-benzophenone conjugates functioned as a sunscreen agent (Heo et al., 2018).

As discussed earlier, the skin has a primary mechanism of protection against UV radiation through the production of melanin (Berneburg et al., 2000). Natural photoprotection of the skin involves also the thickening of stratum corneum (Wulf et al., 2004).

The use of topical retinoids (isotretinoin, tretinoin), antioxidants and α-hydroxy acids has been proven to benefit the treatment of lesions occurred after sun exposure and for slowing down photoageing (Antoniou et al., 2010; Yaar and Gilchrest, 2007).

Enzymes like T4N5 liposomal lotion and photolyase, involved in DNA damage repair (Antoniou et al., 2010; Berneburg et al., 2000) are recommended for preventive or therapeutic interventions for photoaged skin.

It is also recommended the use of protective equipment and the avoidance of sunbeds and tanning booths (Bradford, 2009; Pour et al., 2015; Snowise and Dexter, 2004). Prevention and solar protection is important in the case of newborns and toddlers because the skin is much sensible at this age due to non-existent or underdeveloped mechanisms of molecular protection (Pour et al., 2015).

In case of low temperatures, it is recommended to wear protective clothing, to the number of hours in cold weather, to hydrate to use creams and unguents

to prevent the desquamation of skin and the reduction of the sebum levels (Snowise and Dexter, 2004). Early recognition and early treatment of severe freezing injuries is of great importance because it reduces the tissue loss and other sequelae (Hota and Singh, 2013).

Conclusions

Both the solar radiation and low temperatures present numerous significant effects on face skin.

Because it is almost impossible to avoid exposure to the different factors which affect the face skin, each individual will present, to some extent, the signs characteristic to exposure to environmental factors (temperature, radiations). Because the face skin is directly exposed to solar radiation throughout life, skin accumulates defects of the skin ageing, which tend to accentuate along with excessive exposure at solar radiation.

It is thus necessary a restraint of the skin exposure to these factors along with taking some protection and prevention measures.

Rezumat. Tenul este unul dintre cele mai expuse zone la diferiți factori de mediu ce pot interfera în procesele metabolice localizate la acest nivel sau pot afecta morfologia și anatomia acestuia. Acești factori de mediu influențează unul dintre cele mai importante procese: îmbătrânirea pielii. În această lucrare sunt descrise efectele acute și cronice ale expunerii tenului la câțiva factori de mediu. Factorii care au mare influență asupra fiziologiei și anatomiei tenului sunt razele solare și temperaturile scăzute. De asemenea sunt descrise și câteva măsuri de prevenire a producerii afecțiunilor caracteristice expunerii excesive la soare sau la temperaturi scăzute. Expunerea excesivă la soare cauzează numeroase afecțiuni la nivelul pielii, deși o expunere regulată este necesară pentru buna stare a organismului, precum sinteza de vitamina D. În cazul temperaturilor scăzute, este indicată limitarea timpului de expunere, protejarea prin îmbrăcăminte corespunzătoare a zonelor expuse, evitarea deshidratării, folosirea cremelor și a diverselor unguente pentru prevenirea descuamării și evaporării umidității de la nivelul tenului.

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GALIUM VERUM L. RESTORATIVE EFFECTS IN HEPATIC SUPPRESSION INDUCED BY DARK-RESTRAINT STRESS

Ioana ROMAN[*], Anca D. FARCAŞ[**], Vlad Al. TOMA[**]

Abstract. We studied the protective effects of *G. verum* extract on liver against neuropsychological side effects induced by repetead dark-restraint stress in rats. Experiments were performed on 32 female Wistar rats (120 ± 10 g) which were divided into 4 groups: (1) control group (C); (2) *Galium verum* treated group (E), (25 mg extract/100 g bw); (3) anakinetic stressed group (S) and (4) stress + *G. verum* extract treated group (SE). Exposure to anakinetic stress of the animals resulted in some changes in liver parenchyma structure, characterized by induction of moderate clear dystrophies of the *hepatocytes* which appeared associated with dilation of the sinusoids from around the hepatocytes cords. Liver histology in group E is very similar to that of group C, while in group SE we have reported some discrete granular hepatocytes dystrophy. Histological changes are related to serum biochemistry. Stress exposure determined a significant increase in serum cholesterol levels and a decreased of the activity of AST and ALT whereas *G. verum* treatment induced liver regeneration, based on the histological and biochemical evaluation. Our results suggest that the *G. verum* vegetal extract in a studied dose (25 mg/100 g b.w.) induce positive modulator effects on the dynamics of the entire set of functional and morphological liver parameters.

Key words: *Galium verum* extract; rats, liver; transaminases, anakinetic stress.

Introduction

In Romania there is an ancient folk tradition to use plants as remedies against diseases. The World Health Organization has defined traditional medicine as comprising therapeutic practices that have been in existence for hundreds of years (Usha et al., 2007). Thus, the traditional preparations are consisted of medicinal plants, minerals and organic matter. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy (Hota & Pathi, 2003). It was found that herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. Numerous medicinal plants (*Hypericum* sp., *Lamium* sp., *Calendula* sp., *Trigonella* sp.) and their biological active components are used for the treatment of liver disorders in ethnomedical practice as well as traditional system of medicine in Romania. Many of these plants are evaluated for their hepatoprotective action in light of modern medicine (Khan et al., 2008; Rusu et al., 2007).

In recent decades researches have shown that the basis of many diseases lie on the action of free radicals which, if exceed the neutralizing capacity of the body can become very harmful according to Toma et al. (2017).

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Many xenobiotics such as: alcohol, drugs, pesticides, nitrosamines or organic solvents come in contact with the body and as a result of the metabolism there are produced free radicals that interfere with the main metabolic processes (Rusu et al., 2005, 2007).

Related to xenobiotic mechanism of oxidative stress generation, by its effects, stress may underlie the etiology of many diseases, such as: cardiovascular diseases, diabetes, cancer or neurodegeneration. In response to stress agents, in the body occur a series of changes at the behavior level, neurochemical (Toma et al., 2017) and immunological conditions of prolonged stress which increases the vulnerability of the body to the emergence of various diseases (Farcaş et al., 2018). Long term exposure to increased concentration of corticosterone induced some liver pathological changes based on the disregulation of the functional axis adrenals-spleen-liver and also by corticosterone signaling in hepatocytes, according to Fede et al. (2012) and Trifan et al. (2013). Under various stressful agents, the restraint stress or prolonged anakinetic stress induce the formation of reactive oxygen species (ROS) which causes peroxidation of the lipids, especially to the cell membrane level, with destructive effects on the entire tissue. Prolonged anakinetic stress also induces high levels of corticosterone according to our previously research (Toma et al., 2017) accompanied by the increases of serum cholesterol and lipoproteins - active risk factors in the pathology of cardiovascular diseases and diabetes (Berghian et al., 2011; Nayanatara et al., 2012). An impact of adverse environmental conditions and increased xenobiotic loads tend to result in an influx of the hepatobiliary system diseases (Chen et al., 2015; Francque et al.,, 2016; Mathurin and Bataller, 2015). Since pathological processes in liver are linked to the development of an oxidative stress and an inflammatory infiltration according to Rusu et al. (2007), the complete functional recovery of the liver takes a significant period of time. Hence, the indisputable requirements to hepatoprotective drugs (Hong et al., 2015) are low toxicity and antioxidant, membrane-stabilizing and anti-inflammatory properties (Goryacha et al., 2017). Herbs and other plants that contain more polyphones constitute a class of plants sought because of their possible use in phytotherapy. In our country such herbs are provided from the most popular species of *Galium* with medicinal uses: *Galium verum* L. (*G. verum*) and *Galium mollugo* L. (*G. molugo*). The original phyto-components obtained from the *G. verum* (Lady's Bedstraw) herb raw material meet all the given criteria. Thus, the previous pharmacological studies showed that *G. verum L.* (*Rubiaceae* Juss. Family) has antioxidant, cytotoxic, antimicrobial, neuroprotective and endocrine regulatory effects which were noticed by Al-Snafi (2018). Species of *Galium* L. Genus have long been used as choleretic and anti-inflammatory agents in treatment of the hepatobiliary system diseases.

We proposed to study the protective effects of *G. verum* extract on hepatic disorders induced by repetead restraint stress in rats, in order to obtain phytopharmaceutical preparations used in phytotherapy to protect the liver against neuropsychological side effects.

Materials and methods

Extract preparation

The hydroalcoholic extract of *G. verum* was obtained at the Faculty of Pharmacy from University of Medicine and Pharmacy ``Iuliu Haţieganu`` Cluj-Napoca (RO). The plant material (*Galii veri herba*) was harvested on July 2016 and the species were identified based on a herbarium specimen (CL 666632) at the Botanical Garden ``Alexandru Borza``, Babeş - Bolyai University, Cluj-Napoca, by a specialized taxonomist. Air-dried plants were ground into powder for analysis. The vegetal material was dried at room temperature and then crushed to a fine powder (VI FR X sieve). The fluid extract (1:1) was obtained with alcohol 70% v/v by the percolation technique of Squib (Ionescu-Stoian & Savopol, 1977) using 1-part alcohol for 1-part *Galii verii herba* (pulvis). Qualitative analysis of the polyphenolic compounds (flavonoids, phenyl-propane compounds) of the *G. verum* extract was done by thin-layer chromatography (TLC) by the method proposed of Wagner & Bladt (1996).

The quantitative determination of polyphenols

The quantitative determination of polyphenols (flavonoids, phenyl-propane compounds, coumarin) compounds was carried out by a spectrophotometric method based on the color reaction between polyphenols and Folin-Ciocalteu reagent in an alkaline medium. The absorbance is read at 760 nm, and the results are expressed in gallic acid (Table 1).

Quantitative determination of flavonoids

It was done by a spectrophotometric method based on the color reaction of flavonoids with an aluminum chloride ($AlCl_3$) solution. Flavonoids concentration is calculated using the standard curve constructed on the basis of corresponding absorbance of some rutoside solutions of different concentration (Table 1).

Quantitative determination of phenylpropanoid compounds

The principle of the method is based on the property of phenols to form with nitrous acid nitrosoderivatives which are isomerized in oximes, and due to their weak acid character are dissolved in alkaline solution giving red colorations (Table 1).

Table 1. Concentration of the total polyphenols, total flavonoids, and phenyl - propane compounds in *G. verum* extract.

Extractive solution	Total polyphenols concentration	Flavonoids concentration	Phenylpropane compounds concentration
Fluid extract	1040.8 mg gallic acid/100 g extract	567 mg rutoside/ 100 g extract	341.75 mg caffeic acid/ 100 g extract

Animals

Experiments were performed on mature white female Wistar rats, weighing 120 ± 10 g. Animals were kept under standardized zoohigienical conditions, respectively with free access to pet food (standardized pellet for rodent, Cantacuzino Institute) and water *ad libitum*, standard environmental conditions and 12:12 circadian cycle, in accordance to the Law no. 43/2014 on the protection of animals used for scientific purposes and to the European Communities Council Directive (2010/63/UE).

Experimental design

Animals were divided into 4 experimental groups of 8 animals each, as follows: 1 - control group, (C); 2 - extract group (E), treated for 15 days with hydroalcoholic extract of *G. verum* in amount of 25 mg dry substance from extract/100 g body weight (b.w.), by intragastric gavages, *á jeun*; 3 – dark-restraint stressed group, (S). The immobilization stress was induced in rats by putting them in 20 cm × 7 cm plastic tubes for 3h/day for 15 days by the methods proposed by Marcilhac et al. (1999) and Yokus et al. (2005). There are several 3 mm holes at the far end of the tubes for breathing, which allows ample air but animals will be unable to move. Moreover, animals were kept in dark condition in the period of immobilization to emphasize the stress state; 4 - dark-restraint stress + *G. verum* fluid extract treated group (SE). This group was stressed and received extract of *G. verum* in same conditions as group no. 2. *G. verum* hydroalcoholic extract was administered in an amount of 25 mg extract / 100 g b.w., equivalent to a content of 260.20 µg gallic acid /100 g b.w., 141.75 µg rutozide /100 g b.w. and 85.43 µg caffeic acid /100 g b.w.

In the 16th day, animals were sacrificed by decapitation after deep narcosis with diethyl ether according to our previously studies (Roman & Puica, 2013; Roman et al., 2015). The liver was removed and then processed according to analyzed morphological evaluation and blood was collected for serum biochemical analysis. Blood samples were immediately centrifuged, serum harvested and then frozen in sample vials. It was measured glucose (EPOC Blood analysis system), cholesterol and transaminases (Biomaxima Lublin, Poland, reading with EVOLUTION 2000 Semi-automatic biochemistry analyzer).

Histopathological investigations

The liver was removed and weighed. Fragments of tissues were fixed in Bouin solution for 24 hours; the parts being processed for inclusion in paraffin. The fragment was sectioned at the Reichert - Austria type microtome with a thickness of 7 μm. Fragments were stained with haematoxilin-eosin method for histological structure of liver (Mureşan et al., 1974). The histological examination of the slides was performed with a microscope Olympus BX-51 and the images were captured with a coolsnap-Pro CF color CCD camera (Roper Scientific Photometric, Tucson, USA) using the Image-proplus 4.1 - 4.5 data acquisition software (Media Cybernetics Inc., Bethesda, USA).

Biochemical data were statistically processed by means of Student's „t" test. Aberrant values were eliminated by means of Chauvenet's criterion. A probability value of $p \leq 0.05$ was considered significant.

Results and discussions

Histological study of the liver reveals normal structure of the group C (Fig. 1), with cords of hepatocytes separated by sinusoidal capillaries, bile ducts and supporting tissue. Remack's cell cords have a radial dispozition converging towards the centrilobular hepatic vein, which are separated by sinusoidal capillary showing a discontinuous endothelium. Exposure of the animals to anakinetic stress (S group) resulted in some hepatic parenchymal structure changes, characterized by induction of clear moderate hepatocytes dystrophies, appearance associated with the dilatation of the sinusoidal capillaries lumen around the hepatocytes cords (Fig. 2). In group E the histology of the liver is very close to the group C (Fig. 3), while in the group ES several aspects of a clear and granular discrete hepatocytes dystrophy are observed (Fig. 4).

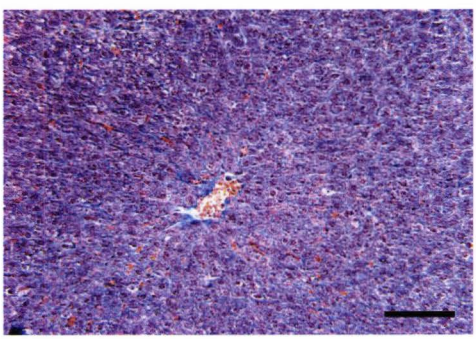

Fig. 1. Normal aspect of hepatic parenchyma structure in group C, (x 200). Scale bar, 20 μm.

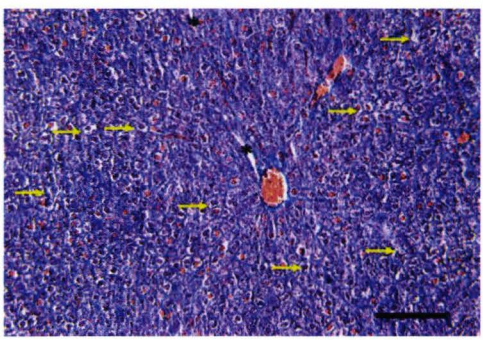

Fig. 2. Moderate clear hepatocytes and granular dystrophy (yellow arrows) and dilation of sinusoidal capillaries lumen (black asterisk) in group S, (x 200). Scale bar, 20 μm.

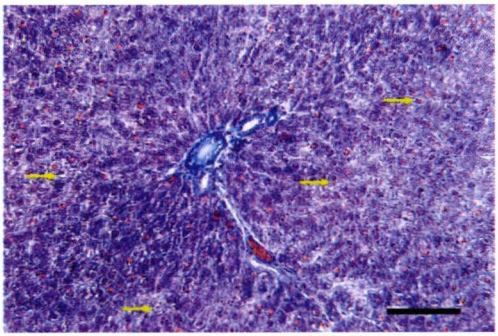

Fig. 3. Aspect close to normal of hepatic parenchyma in group E, (x 200). Several clear distrophyc aspects are observed (yellow arrows). Scale bar, 20 µm.

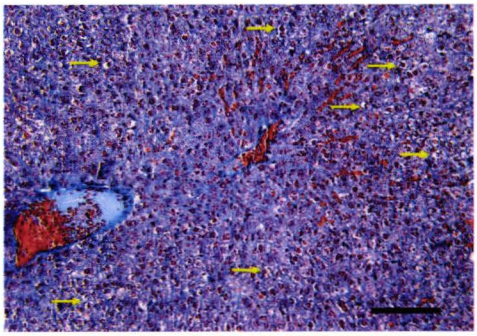

Fig. 4. Aspects of a clear and granular discrete hepatocyte dystrophy (yellow arrows) in group SE, (x200). Scale bar, 20 µm.

The results of the glycemia, serum transaminases (AST and ALT) and the total serum cholesterol determinations are presented in Table 2.

Table 2. The blood sugar, serum cholesterol and serum transaminases (AST and ALT) level in the four experimental groups (C, E, S and SE)

	C	E	S	SE
Glycemia (mg/dL)				
x ± SD	153.83 ± 5.20	161.83 ± 3.92	178.8 ± 5.96	166.6 ± 2.39
p	-	> 0.25	< 0.02	< 0.05
D%	-	+ 5.20%	+16.23%	+ 8.30%
Cholesterol (mg/dL)				
x ± SD	140.19 ± 10.05	155.8 ± 6.17	137.67 ± 13.33	170.75 ± 4.35
P	-	> 0.25	> 0.5	< 0.02
D%	-	+ 11.13%	- 1.79%	+21.79%
AST (U/dL)				
x ± SD	207.37 ± 10.79	223.17 ± 17.74	167.87 ± 12.68	167.87 ± 3.64
p	-	> 0.5	> 0.05	< 0.02
D%	-	+ 7.62%	- 19.04%	-19.04%
ALT (U/dL)				
x ± SD	72.63±3,43	73.62 ± 337	63.9 ± 4.28	54.45 ± 2.87
p	-	> 0.5	> 0.25	< 0.01
D%	-	+ 1.36%	- 12.01	-25.03%

Note: C = control group; E = extract group; S = dark-reistrant stress group, SE = S + E group. Are given: percentage difference vs. the control group (±D %); mean values homogeneity (x) ± SD (standard deviation), ± D% was considered to be statistically significant for p = 0.05.

Analysis of the results show that the blood sugar level is increased significantly (16.23%, $p < 0.02$) in S group compared to the C group. In the SE group was observed a moderate increased in blood glucose concentration in the limit of statistical significance, (8.30%; $p < 0.05$), while in the E group the value of this parameter increases only 5.20% as compared to controls.

The serum cholesterol levels in animals treated with *G. verum* extract (group E) is insignificant increased (+ 11.13%) and in the SE group the serum cholesterol levels significant increase with 21.79% (< 0.02) compared to the group C. In the S group, cholesterol level was with normal concentration (140.19 ± 10.05 mg/dL) for rat standard biochemical values (Farcas et al., 2018).

The activity of serum transaminases – AST and ALT undergo some changes according to the treatment applied to the animals. Thus, in the E group, treated only with *G. verum* plant extract, there was a slight increase without statistical significance both the AST (+ 7.62%), and the ALT activity (+ 1.36%) compared to Control. In contrast, in S group - subjected to anakinetic stress, the activity of AST significantly decreases (-19.04%; $p < 0.05$) also ALT value, but insignificant (- 12.01%; $p > 0.25$) compared to the Control group. Administration of the *G. verum* plant extract following to exposure of the animals to anakinetic stress (SE group) has resulted in a statistically significant reduction in both AST (- 19.04%; $p < 0.02$) and ALT (- 25.03%, $p < 0.01$) activity compared to Control.

Discussion

Histological examination of the liver didn't reveal significant structural morphological changes of the hepatic parenchyma in the three experimental groups compared with the control group. However, in S group, histological aspects illustrated by the clear hepatocytes moderate dystrophy associated with dilation of the sinusoidal capillaries lumen didn't have pathological character, changes being reversible and temporary. The clear hepatocytes moderate dystrophy, which occurred following the administration of the plant extract to the group E and the administration of the plant extract under stress exposure (group ES) could be the expression of endoplasmic reticulum and mitochondrial changes (Lieber, 2000). These changes are not so pernicious, having a temporary and reversible character (Michell & Contan, 2003). On the other side, liver atrophy may have multiple causes, among which is the poor nutrition, viral infections, reducing the amount of blood, loss of hormonal support, xenobiotics exposure or loss of the nervous contribution to the target organs. As it is known, the liver plays a central role in the biosynthesis, metabolism, clearance and body defense reaction. These functions require a sufficient nutrient infusion and oxygen supply which is closely dependent on an adequate microcirculation (Vollmar & Menger, 2009). The main regulators of

the hepatic sinusoidal blood flow are present in the portal venous system according to Kinoshita et al. (1995) and Oda et al. (2003). In the SE group, the histological lesions of the liver are comparable with the results obtained by Abdul-Razzak (2012), who achieved similar results in rats supplemented with 500 mg vitamin C/kg/day and subjected to psychosocial stress, as well as those of Kollmar et al. (2007), who reported the presence of sinusoidal dilatation and increases of parenchyma cell vacuolization due to the initial decrease in the liver portal blood-deprived liver (PBL) microcirculation in BALB/c mice. Histological study in the SE group, suggests stimulation of HHS axis activity. Thus, hypothalamic CRH release induces stimulation of secretion of ACTH, which then acting on the adrenal cortex and increases the corticosterone secretion. In turn, increased plasma corticosterone affects the mobilization of stored fat and carbohydrate reserves, which increase blood sugar levels, as it happened in our experiment. Increased levels of corticosterone and glucose can be restored by anti-stress agents as was demonstrated by Meera (2009) or Rather et al. (2013). The results of liver histological analysis showed that administration of *G. verum* extract in the animals exposed 15 days to the immobilization stress, gave rise to liver moderate changes, including mild to moderate degeneration, sinusoidal dilatation and hepatocellular individualization. These changes were reversible and harmless.

Biochemical evaluation depicted that animals' exposure to a restraining for 15 days (group S) resulted in a significant increase in glucose levels compared to Control. The increase in blood sugar levels is typical to stress and could be due by decreasing of insulin secretion associated with a release of glucocorticoid hormones (Zardooz et al., 2006). The high level of glucose in blood can be achieved by two mechanisms: either by supporting the liver gluconeogenesis from amino acids or by inhibiting the growth of hepatic glucose concentration and utilization by peripheral cells (Nayanatara et al., 2012). In our experiment, in the group E, as well as in the group SE, glucose level is low compared to the group S, result which is comparable to that achieved by Rather et al. (2013), who demonstrated that administration of gallic acid (a polyhydroxyphenolic compound which is characteristic for *G. verum*) obtained a significant reduction in blood glucose levels in rats subjected to restraint stress for 21 days. The level of serum cholesterol in the group subjected to the immobilization stress for 15 days (group S) did not show significant changes compared to Control, and this result is registered by Rostamkhani et al. (2012). The administration of the *G. verum* plant extract in group E resulted in a moderate increase of serum cholesterol, while the animals treated with the plant extract and exposed to anakinetic stress (group SE) had a statistically significant increase in serum cholesterol levels. Analysis of the results leads us to assume that high cholesterol concentration may also occur due to increased concentration of HDL fraction by gallic acid, a bioactive

compound comprised in *G. verum* extract used in our experiment. Similar results were obtained by Rather et al. (2013), who observed that administration of gallic acid at a dose of 10 mg/kg causes a significant increase in HDL compared to the group exposed to immobilization stress for 21 days. As pointed out above, in the SE group treatment-induced liver damage are more pronounced than in group S. In the group treated only with the extract, no liver damages appear and no significant changes in serum transaminases levels. The fact that the level of serum transaminases is lower in group SE can be correlated with experimental results obtained by Abdul-Razzak et al. (2012) and Ozdil et al. (2004), suggesting that lowering serum transaminases would be due to alterations in the lipid and protidic metabolism caused by hepatocytes dystrophy and also to the fact that antioxidants, such are phytophenols from *Galium* extract, are essential to prevent free radicals and free radical lipid peroxidation formation

The biochemical parameters considered show the possible mechanism of the hepatoprotective effects of the *G. verum* extract and can be explained by an inhibited cytolysis of the hepatocytes and reduced oxidative stress, which is indicative of the membrane-stabilizing and anti-inflammatory properties of the *G. verum* principal components according to Goryacha et al. (2017). Hepatic disorders induced by repeated restraint stress in rats were diminished by *G. verum* treatment and our assumption was confirmed by the obtained experimental results.

Conclusions

Histological examination of the liver didn't show significant liver morphological changes in the experimental groups compared with the Control. The obtained biochemical changes are related to the morphology of the liver. Also, it was established that the extract of *G. verum* L. herb in a dose of 25 mg/100 g display a hepatoprotective activity against the dark-restraint stress-induced hepatic disorders in rats. This was evidenced by the improvement of all assessed biochemical parameters and histopathological analysis. The results of the studies give grounds for further pharmacological research concerning the hepatoprotective activity of the *G. verum* extract.

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Rezumat. Am studiat efectul protectiv al extractului de *G. verum* asupra ficatului împotriva efectelor secundare neuropsihologice induse de stresul de întuneric şi imobilizare la şobolani. Experimentele s-au efectuat pe 32 de şobolani Wistar femele (120 ± 10 g) care au fost împărţite în 4 grupuri: (1) lotul martor (C); lotul tratat cu extract de *Galium verum* (E), (25 mg extract/100 g animal); (3) lotul stresat anakinetic (S) şi (4) lotul stresat şi tratat cu extract de *G. verum* (SE). Expunerea la stress anakinetic a animalelor a determinat unele modificări ale parenchimului hepatic, caracterizat prin inducerea de distrofii clare ale hepatocitelor care apar asociate cu dilatarea sinusoidelor din jurul cordoanelor hepatocitare. Histologia ficatului la lotul E este asemănătoare cu cea a martorului în timp ce la lotul SE s-au evidenţiat unele distrofii discrete granulare ale hepatocitelor. Modificările histologice sunt în corelaţie cu cele biochimice. Expunerea la stres a determinat o creştere semnificativă a nivelului colesterolului seric şi o scădere a activităţii AST şi ALT, în timp ce tratamentul cu extract de G. verum induce regenerarea ficatului, cum s-a văzut din evaluarea biochimică şi histologică. Rezultatele noastre sugerează că extractul vegetal de G. verum în doza studiată (25 mg/100 g animal) induce efecte modulatoare pozitive asupra dinamicii întregului set de parametri funcţionali şi morfologici studiaţi.

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THE NUTRITIONAL REQUIREMENTS FOR THE RACE HORSES

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Abstract. The feeding and nutrition of horses trained for performance are much more complex than the feeding of other classes of animals. This is due to the stress, the effort to which they are subjected. Unfortunately, it is still an incomplete topic, because there is little information with regard to the optimal nutritional requirements of the horse. Actually we use the information obtained with other animal species or human athletes, or the information acquired in the competition field in order to help a program formulation concerning the feeding and nutrition of performing horses. This information can be used until we get accurate information from the actual research which can improve the program being watched. In a way, a trained horse is like an athlete whose nutritional requirements are very high, but not very well known. That is why, it is not surprising that only a small percentage of pure-bred trained horses are still healthy after a competitive year. This paper aims to describe the nutritional requirements of horses on the path of performance, and the relationship between the addition of plants and their mode of selection by different criteria, the nutritional value offered by the plants being mirrored in its preferences.

Key words: nutrition, behavior, horse, vitamins, feed.

1. High performance equine nutrition

The art and science of feeding remain important for the proper nutrition and represent a superior quality of horses' performance at a high level. Animals' nutrition is a vital component of horses' care. Without a balanced diet and proper management of the feeding stuffs, various health problems may appear (Mastellar et al., 2018).

The behavioral phenomenon is distinguished by its complexity, primarily expressed through the interpenetration of what the natural ability is and what is acquired in the course of the existence of the individual. By the triggering of a specific behavior of the animal seeks to meet a series of requirements: hungry, Thirsty, or seek to avoid a situation which jeopardizes the existence (Chelaru, 2004).

Most current levels of nutrition are based on studies of horses kept in the stables or in the experimental conditions, which are not similar to those of the performance horses.

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The stress of the heavy exercise as well as the weight of a horse rider, increase the need for certain nutrients for a competition horse (Hoffman et al., 2009). The performance horses submit more musculature, being fed with a large quantity of cereals concentrates, and the meals are strictly scheduled three times per day (Bruschetta et al., 2018).

The training sessions involve the increasing of muscle mass, the stabilization of bones, of ligaments and tendons. Thus the necessary energy to support the functioning of the cardiovascular and circulatory systems, which are important for muscle performance, for the digestive system as well as for the body cooling effect with a proper sweat production is provided (Paz-Silva et al., 2014). Dietary supplements are commonly used by horse owners, to provide an additional source in their diet. Recent studies have analyzed specific types of supplements in order to improve horses' performance and the prevention of joint diseases (Murray et al., 2018).

Not only body mass but also the physical performance, the competitiveness and image of the horse are considered a reflection of energy intake. The following criteria are important in shaping the ration of a competitive horse:

- The energy provided must match the horse's fitness and exercise requirement.
- The intake of protein-quantity and quality is more important during the periods of muscle growth.
- It is necessary to feed the horse as required by nutrients and vitamins.
- The high and continuous performance requires portions of roughage which is a lower limit on quantity - and that's rather the exception. The roughage must not exceed 0.5 kg per 100 kg of body weight (Cunha, 2004).

A horse that is trained to compete and learn some commands should be treated like a human athlete. Both horse and human must be in an excellent shape to compete effectively and win. A man has the advantage of training and competing at an older age, while the horse competes at a relatively early age. If a man has a lifetime of 77 years and the horse has a lifetime of 22 years, this means that a 2 years old horse is comparable to a 7 years old person. Most of horses begin their training at the age of one year old, which is comparable to a boy who starts training himself at the age of about three years old. Therefore, the horse is in training at an early age, as compared to man. This means that they put so much stress on the feet, body and skeletal system of young horse, which is still quite immature. This explains some of the conditions that occur in the horse's legs, especially the tendons. They are trained starting from an early age so that physiological legs and their bodies are not developed well enough to take the stress of training and performance (Cunha, 2004; Paz-Silva et al., 2014).

High performance success doesn't reside only in feeding. A good education and a proper training of the horse as well as its talent are still considered the conditions for success. The feeding requirements (according to the horse's age,

race and physical condition) must match with the horse's constant training until it gets the high performance level. Actually, a high performance horse must produce and store glycogen in its muscles for a quick access of it during the competition. That's why its diet and exercise are so important because they should help in producing it. During horseracing which involves high speed (or during some jumping movements) the energy extraction is carried out in the anaerobic area with a high loading of the energy balance and of the metabolism (Pirkelmann et al., 2012).

The degradation in horses legs which may occur indicates the need for research information to reduce these losses. It is estimated that only 50% of race horses get on the racetrack and only 20% of them remain healthy in the first year of racing. This indicates that it is very important to learn how to feed a horse to minimize and if possible to eliminate these costly losses (Cunha, 2004).

2. Background of the horses digestive system

The horse's digestive system or digestive tract, is a tube which length is of about 100 cm from its mouth to its anus with the associated organs (Fig. 1). These include the mouth, the esophagus, the stomach, the intestines, and other organs, such as the liver, the teeth, the pancreas and the salivary glands. The digestive tract can be divided into two functional parts, as can be seen in (Fig. 1): foregut (the anterior part of the tube from the mouth to the intestine) and hindgut (the rear of the digestive tract including also the rectum and the large intestine). The rear part of the digestive tract has some similarities to that belonging to the ruminants because the microbial action is not very big and the absorption of nutrients is not so effective because the cecum is located in the posterior side of the small intestine (Cunha, 2004, Joe and Pagan, 2009). As a result the horse is between pigs and ruminants in the process of digestion of food, this taking place in the intestine under the influence of microsimbions and cellulolytic bacteria.

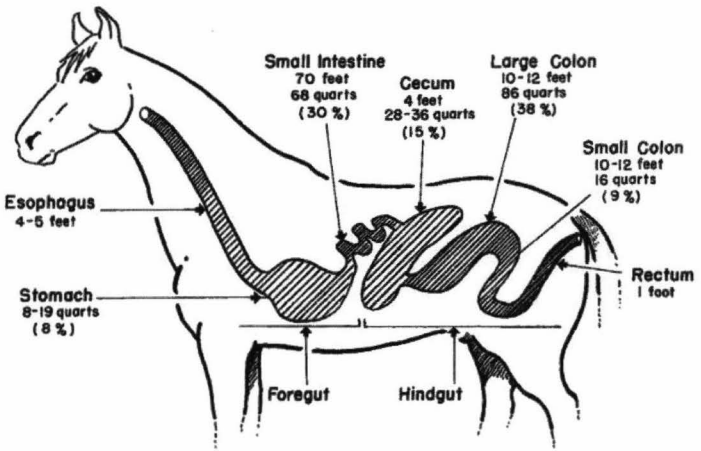

Fig. 1. Horse's digestive tract (after Cunha, 2004)

Horses cannot vomit due to specific anatomical features. Thus, on the entry into the stomach (called "cardia") there is a muscular formation in the form of a "Swedish tie knot" whose shrinkage does not allow the animal to vomit. The large intestine has a large capacity (of 120-160 liters), the cecum is bulky (of 35-40 liters) and the liver (three-lobed) is devoid of the gall bladder (Acatincăi, 2003).

3. Nutrition requirements

Nutrition requirements imply the science of medical and biological relation between nutrients (energy, plastic and growth stimulators) of the body and sources to meet these requirements in all their complexity (Nichita, 1984). The main manifestations of the relationship between animals and food in point of phylogenetic formation are the followings:

-food (as a source of nutrition) determines the metabolism which is the essential function of the living matter and the existence of life

-food (as a carrier of pathogenic agents) which can produce various morbid conditions affecting the physiological vital and productive functions of animal's body

Providing the corresponding power level to satisfy nutritional requirements of livestock genetic parameters productive performance is determined by the relation between the animal and its fodder.

3.1. Vitamins

Vitamins are organic compounds. They differ in structure and function. They are not linked together; do not form complex structures such as proteins, fats and carbohydrates. Vitamins are required in very small quantities. They perform many important functions in the body. Without vitamins, a horse cannot grow, reproduce, or to perform work. It is very important that all vitamins are supplied in adequate amounts in the diet of the horse. Nutrient content in vitamins is differentiated depending on the nature of the food, the conditions of fodder's preservation and storage. Unfortunately, there is a lack of experimental information about the vitamins the horses need. Moreover, it is not known yet which are the vitamins which should be added to a horse's balanced diet (Cunha, 2004).

Vitamins are of two types: soluble and fat soluble. Water soluble vitamins include vitamin C and the B vitamins (thiamine, riboflavin, choline, biotin, vitamin B6, vitamin B12). Fat-soluble vitamins include vitamin A, vitamin D, vitamin E, vitamin K. The vitamin sources are:

a) Forage carrots, are a valuable and constant source of carotene. Vitamin-factors A, vitamin E, C, K and some B vitamins may be provided in the green fodder.

b) Green plants (green, fibrous roots) are valuable sources of vitamins B2, B5, B1 and B10. Vitamins which cannot be entirely provided by the fodder are supplemented with synthetic vitamins. Natural vitamins are better tolerated by animal's body than the synthetic ones (Nichita, 1984).

a) Vitamin A

Vitamin A is one of the fat soluble vitamins. This does not occur in hay or pasture, but the carotene is present and changed into vitamin A in the intestinal wall of the horse (Ott et al., 1989; Cunha, 2004). Vitamins accumulate in green pastures, corn and carrots. Performance horses need vitamin supplements: mineral mixtures in nutrition through special supplements or special dietary vitamins and minerals, which may be even injected (Combs and McClung, 2017).

Deficiency and effects

Vitamin A deficiency can besensed at horses which are fed only with hay or are kept on drypastures for long periods of time. Vitamin A causes certain effects such as: anorexia (loss of appetite), poor growth, night blindness, lacrimation, joint damage, keratinization of the cornea, skin and progressive weakness (Cunha, 1988; Donoghue and Kronfeld, 1980; Combs and McClung, 2017).

b) Vitamin D

All types of food are very low in vitamin D. Horses get vitamin D from the sun (sunlight changes in the skin vitamin D sterols).During summer horses assimilate all the vitamin D they need from rays sun. In winter the action of sunlight to produce vitamin D is lower (Pirkelmann et al., 2012). Vitamin D is necessary for both absorption and utilization of Ca and Fe in developing and maintaining strong bones. Therefore it requires an adequate level of vitamin D to maintain the posture (Uhl, 2018).

Deficiency and effects

The less time horses spend outdoors the more they suffer of vitamin D deficiency. The lack of vitamin D can lead to problems such as: softness bones, bone deformities, gait stiffness, frequent cases of fractures (Cunha, 2004; Uhl, 2018).

c) Vitamin K

Normally horses synthesize vitamin K in the intestinal tract. Certain factors in the food or drug treatment may decrease the synthesis of vitamin K. The vitamin present in the diet of the horse can be measured by determining the time of flocculation of the blood. Blood clotting time is very important in case of injury or if surgery is needed (Pirkelmann et al., 2012).

Deficiency and effects

Vitamin K deficiency leads to low levels of prothrombin which increases the risk of clotting and bleeding (blood does not coagulate) resulting in strong anemia (Cunha, 2004; Combs and McClung, 2017).

Water-soluble vitamins. This category includes vitamin B complex and vitamin C.

Vitamin B1, known also as thiamine, has an essential role in regulating metabolic processes (in particular, the metabolism of glucose), in nervous and endocrine activity, in activating insulin action. Since segmentation of carbohydrates is increased during the performance, it is important that the thiamine should be available in sufficient quantity (Haţieganu et al., 1984).

Deficiency and effects

Horses fed with a law-quality hay have developed a thiamine deficiency which has risen endemic effects. Deficiency manifests itself in nervous disorders, loss of appetite and body mass (anorexia nervosa), disorders in reproduction, the discordance (in particular the hind legs), a decrease of thiamin in the blood increase pyruvic acid in the blood and heart dilated (Cunha, 2004).

d) Vitamin B2

Also known as vitamin G or riboflavin, Vitamin B2 is a fundamental component of animal tissues necessary to ensure vital functions and growth. It has an important role in ocular accuracy and influences reproduction (Haţieganu et al., 1984).

The amount of riboflavin excreted in urine is closely linked to its contribution. Rapid excretion of excess riboflavin may result in lack of toxicity in the body (Cunha, 2004).

Deficiency and effects

The tendency of not allowing horses stay on pasture resulted in decreased levels of riboflavin in horse nutrition. Freshly cut grass and meadows of high quality are an excellent source of riboflavin. Riboflavin is an essential enzyme necessary for the use of the fats, carbohydrates and proteins in the body. Deficiency of vitamin B2 causes a low growth rate (Cunha, 2004).

e) Vitamin B6

Vitamin B6 is also referred to as pyridoxine or as vitamin H. It is synthesized in the intestinal tract. The largest amount is in the cecum and large intestine. Vitamin B6 works as part of the enzyme system and is therefore a very important vitamin. It is concerned with the metabolism of proteins, fats carbohydrates and also intervenes in the metabolism of tryptophan (Haţieganu et al., 1984).

Deficiency and effects

Without vitamin B6, tryptophan cannot be used by the animal. Deficiency is manifested by loss of appetite, growth retardation, loss of the hair, nervous system disorders, tongue injuries and fissured testicles (Cunha, 2004).

3.2. Minerals

Animal's health and productivity show an example of dependency and understanding of the very complex significance within the biological chain

between soil, plants and animals (Miloş and Drînceanu, 1980; Dominguez-Vara et al., 2017).

The plants do not require selenium or iodine and can grow normally and produce optimal yields for animals, even if they contain an insufficient amount of these two minerals (Miloş and Drînceanu, 1980).

The mineralization of the food is influenced by a number of peculiarities of the plant, including the signals of genetic differences, botanical composition, the growing stage, the various parts of the plant, the chemical form of the mineral salts, the climate, the season, etc. In general, it decreases with increasing content of plants growing in K, Na, Cl, Co, Zn and increases in Si, Mg and Ca. Young plants contain the most assimilable forms. Phosphorus in hay is of 60%, while in green plants, the content is 2% (Miloş and Drînceanu, 1980).

Sodium is essential to certain plants. Rarely, the plants suffer from a sodium (Na) and chlorine (Cl) deficiency. The food does not contain the commonly used sodium enough to meet the needs of the animal's body. It is therefore necessary to supplement food with these minerals (Cunha, 2004).

Minerals are necessary for the maintenance of bones, teeth, but they also serve the whole body. Almost every animal body process depends on the proper functioning of each mineral. They are very important in the growth, reproduction and lactation (Grimwood et al., 2016). The most important (essential) minerals are:

a) Calcium (Ca). It is estimated that approximately 98% of Ca is in the bones and teeth, while the remaining 2% is involved in the very critical functions. It has many roles in normal blood clotting involved in neural tissue and muscle necessary for the heart (contraction) and enzymatic activation. Some enzymes are involved in the activation and secretion of hormones. Calcium and phosphorus represent half of the minerals in milk and half of the minerals in horse's body (Pirkelmann et al., 2012; Toribio, 2011).The lack of Ca leads to rickets characterized by weak mineralization of osteoid, the joints, the weakening and deformation or fracture of horse's bones (Toribio, 2011).

b) Phosphorus (P). It is found in about 85% just as Calcium in bones and teeth. It is an important part of many enzyme systems. It is an essential component of organic compounds. It has an important role in muscles, nerve tissue, skeleton and the normal structure of blood. Therefore, the absence of phosphorus affects all aspects of horse's life. (Cunha, 2004; Maly et al., 2018). The mineral content of grains (barley, oats, maize, wheat) and hay varies depending on the stage of maturity, the level of exposure to sunlight and the soil type (Cunha, 2004).

c) Magnesium (Mg). It is an essential macro element, and approximately 60-70% of it is found in bones (a bone contains approx. 0.75% of Mg), and the rest of 30-35% is contained by body's fluids and soft tissues. Dairy products

may increase the absorption of Mg. Magnesium is more available in milk than in flaxseed flour (Cunha, 2004; Stewart, 2011). The symptoms of magnesium deficiency include irritability, muscle tremors, sweating and, in some cases, death. (Stewart, 2011).

d) Sodium (Na). This is important for the water balance, heat and acid-base interaction in the body. The physical effort makes the body lose significant amounts of sodium. Especially after sweating additional sodium is required. It depends on exercise, fitness and ambient temperature. (Cunha, 2004).

The effects of sodium deficiency are: thickening of the intestinal content, reducing the buffering capacity of the cecum (constipation, harmful fermentations) ingestion of grass roots, heat stress and slimming. But a negative effect is also considered a higher sodium intake, having the following consequences: higher water ingestion and diarrhea, which is a problem especially in infants foals and young horses (Pirkelmann et al., 2012).

e) Potassium (K). It maintains optimal health and normal operation of the system, pH of blood and other body fluids and it must be maintained within a narrow range. The requirement of potassium in food varies between 0.3 to 0.43%. The biggest requirement is 0.43% and occurs during intense work (Cunha, 2004). Lack of K leads to loss of appetite, muscle weakness which leads to poor performance, stiffness and paralysis, heart abnormalities, nerve disorders (Pirkelmann et al., 2012).

3.3. Carbohydrates and fibers

Carbohydrates include sugars, starches, cellulose, lignin and related substances. They are mostly food. The term food fiber refers to carbohydrates and cellulose that forms the cell walls of the plant (together with lignin). Food having a higher fiber content are much easier to digest (Cunha, 2004). Cellulose is digested by microorganisms in the digestive tract, especially in the cecum and colon. The fiber content of the early cut hay is more palatable than the fiber obtained from the more mature grass. Fiber from the green pastures is more digestible than dry, more mature grass (Cunha, 2004).

Lignin, largely grown in hay, dry pastures, is not digestible by the horse; it reduces the digestion of cellulose and other nutrients from the plant nutrients. The nutrients are inside the cell walls (Cunha, 2004). To create optimal conditions for recovery of the food some features of horses must be taken into account (they are grazing, ruminants, having a mono-gastric digestive tract with small capacity). They digest cellulose and the other nutrients with much cellulose in the food at a lower level (Cunha, 2004). Young horses, race or performance horses need hay or pastures that are low in cellulose and high carbohydrate content.

3.4. The proteins

It generally refers to the crude protein, which is defined as the content of azote in horses' food. The protein consists of a unique mixture of several amino acids combined together; each protein has a different and unique amino acid composition. They are not necessary in food as such, but they can be an essential source (Cunha, 2004).

Animals continue to use the proteins, either to produce new tissues, as well as in growth and reproduction or to repair damaged tissues. Horses require a rich, regular protein intake to prevent a reduction in growth or a weight loss. The protein will be taken in certain tissues to maintain vital functions of the body tissues as long as possible (Hodgson et al., 2014).

Proteins are required for the formation of milk, muscles, skin, hooves, hair, hormones, enzymes, cells of the body constituents (Cunha, 2004; Hodgson et al., 2014). A balanced diet consists of a mixture of several types of protein (feed, grain, hay have different types of proteins). Protein consumed by horse is digested and turned into amino acids in the digestive tract (Cunha, 2004; Bachmann et al., 2018). The amino acids are placed together in various combinations to form proteins and the proteins are often referred to as blocks. Amino acids are part of two categories: some are essential, others are dispensable (Hodgson et al., 2014).

Indispensable amino acids are synthesized at low levels or not at all by the body, therefore a certain amount must be provided for the food. This means that they are an essential part of the food and should be included in a suitable diet for horses according to their needs (Cunha, 2004; Hess, 2015).

Dispensable amino acids are named so because they can be synthesized in adequate amounts in the body and should not be taken from an external source (Cunha, 2004; Hess, 2015).

3.5. Water

Although water is ingested less than food, water is an important resource for horses. They present requirements for not only the quantity and quality of water, but drinking water must be always available (Pirkelmann et al., 2012). A horse ingests water by immersing the lips almost closed below the water surface and by suction action draw water into his mouth. A watering action consists of a series of gobs. The horse then stops to look around and then maybe drinks some more. Thus, during a stop at the water source, they can consume 4 liters of water or more (Butudom et al., 2002). Water demand is dependent on many factors. The food can have different percentages of water content, the higher (about 80% weed) or low (about 14% hay) (Pirkelmann et al., 2012).

Many water sources are used for drinking, but they must meet the condition to be deep enough to allow immersion of horses' lips. Small ponds resulting from precipitation or springs are usually used by many horses let free.

Sometimes a horse creates its own source of drinking by digging in the ground with its foot. Snow and juicy food intake can diminish water demand (McDonnell, 2003). Watering can take place both day and night on a fixed schedule. When the water is easily accessible, horses consume small amounts several times in one day (Gill et al., 2011). Moreover, watering is of great significance to sweat, milk, urine, feces, breath and depends on the proper use and the ambient temperature. Watering for hot horses or when horses were deprived of water implies a pre-cooling action and the water is provided only in small amounts (Pirkelmann et al., 2012).

A horse behavior often met is the wet hay meals (Fig. 2). It is generally concerned with dried fodder. Thus before chewing it, the horse gets some hay and then soaked it in automatic watering device. This behavior has been adapted for better digestion and delivery of food flavors before swallowing.

Fig. 2. Wetting hay meal

4. Types of food

4.1. Horse's green food

It is provided by green meadows, by different crops of fodder and occasional sources. It is rich in protein substances, in vitamins and minerals, and it can be considered complete food from this point of view. Having a high water content (of 50% -85%) the green fodder is a succulent, diuretic and healthy diet for the animals which consume it with pleasure. Unlike other fodder, green fodder is rich in vitamin A and other vitamins. Green fodder produced by natural grasslands can be used by grazing, by mowing it or in the form of hay (Haţieganu et al., 1984). The nutritional value and production of the fodder are determined by the association of the vegetation flora in the grasslands (Table 1). In terms of nutrition, natural plants on the fields are grouped into: high nutritional value grasses, average nutritional value and low nutritional value grasses which are more difficult to be consumed by animals (Haţieganu et al., 1984).

Table 1. The main groups of plants in the botanical composition of natural grassland

SCIENTIFIC NAMES	COMMON NAMES
High nutritional value grasses	
Agropyron repens	Wheatgrass
Agrostis vulgaris	Bent
Alopecurus pratensis	Foxtail
Arrhenatherum elatius	False oat-grass
Bromus erectus	Erect brome
Cynosurus cristatus	Crested dog's-tail
Dactylis glomerata	Cocksfoot
Festuca pratensis	Meadow fescue
Festuca rubra	Red fescue
Lolium perenne	Ray grass
Poa pratensis	Bluegrass
Average nutritional value grasses	
Agropyron cristatum	Crested wheatgrass
Agrostis tenuis	Bent
Lolium multiflorum	Ryegrass
Poa alpina	Alpine bluegrass
Poa bulbosa	Bulbous bluegrass
Phleum pratense	Timothy grass
Trisetum rupestris	Kveinhavre
Agrostis rupestris	Bent
Anthoxanthum odoratum	Holy grass
Echinochloa crus-galli	Barnyard grass
Festuca ovina	Sheep fescue
Poa nemoralis	Wood bluegrass
Poa trivialis	Rough bluegrass
Phleum phleoides	Purple-stem cat's-tail

Low nutritional value grasses	
Andropogon ischaemum	Yellow bluestem
Brachypodium pinnatum	Heath false brome
Cynodon dactylon	Bermuda grass
Deschampsia caespitosa	Tussock grass
Festuca sulcata	Fescue
Nardus stricta	Matgrass
Phragmites communis	Reed
High nutritional value legumes	
Lathyrus pratensis	Meadow pea
Lotus corniculatus	Trefoil
Medicago sativa	Lucerne
Onobrychis viciaefolia	Sainfoin
Trifolium hybridum	Alsike clover
Trifolium pratense	Red clover
Trifolium repens	White clover
Average nutritional value legumes	
Medicago falcata	Sickle alfalfa
Medicago lupulina	Hop clover
Trifolium fragiferum	Strawberry clover
Vicia angustifolia	Beans
Vicia cracca	Beans
Lotus tenuis	Narrowleaf trefoil
Trifolium montanum	Mountain clover
Trifolium pannonicum	Hungarian Clover
Vicia pannonica	Beans
Low nutritional value legumes	
Anthyllis vulneraria	Kidneyvetch
Glycyrrhiza glabra	Liquorice

Melilotus albus	Honey clover
Genista tinctoria	Dyer's greenweed
Other edible plants	
Achillea millefolium	Yarrow
Amaranthus retroflexus	Tumbleweed
Atriplex tatarica	Orache
Carum carvi	Caraway
Cichorium intybus	Chicory
Plantago lanceolata	Ribwort plantain
Potentilla alba	Cinquefoil
Polygonum aviculare	Knotgrass
Taraxacum officinale	Dandelion

The grazing is a very important element in increasing the production of grass, steered grazing being the cause of degradation of vast tracts of grassland. Grazing rationally directed is the most indicated. It overcomes the shortcomings and promote an increasing production of grass. Lately, limited grazing of about 3-4 hours per day with pasture and by grass chute feeding is mostly used (Haţieganu et al., 1984; Fleurance et al., 2016).

Table 2. Average consumption by grazing and grass chute feeding (Haţieganu et al., 1984)

	Type of consumption	
	By grazing	By mowing and grass chute feeding
Cattle		
Cows	50-70	40-50
Up to 1 year old	15-20	10-15
Calves Heifers	30-40	25-30
Bulls Oxen	20-40	20-30
Equines		
Mares	40	30-35
Foals	30	15-25
Traction horses	20-40	15-30
Sheep		
	8-10	-
Pigs		
	6-7	4-5

Green grown fodder constitutes the main source of fodder. As you can see in this table (Table 2) equines have a slightly different approach from the other animals in the management of food intake. In order to cultivate and obtain green fodder, green pulses and perennial grasses which provide annual high yields rich in nutrients must be chosen.

a) Perennial Legumes

Lucerne (*Medicago sativa*) is a valuable plant, having a high content of protein, minerals and vitamins. They are found in a larger quantity than in flower leaves and stems. It exceeds other green fodder not only by the protein content, but also in terms of the biological value. The protein content, cellulose and calcium increase in half while the phosphorus content is not influenced by the growth phase. Lucerne, as pasture, is used until it reaches the flowering stage. After flowering, it produces digestive disorders due to the increased saponin content. Lucerne is provided on average of 20-25 kg in a horse meal. Because lucerne is rich in carbohydrates, it can cause bloating (Haţieganu et al., 1984).

Red clover (*Trifolium pratense*), white clover (*Trifolium repens*) are the main pulses which can cause bloating. The record high production appears in wet and cool regions. Its cultivation significantly contributes to soil fertilization; it is mainly used in the production of feed (McKenna et al., 2018).

Sainfoin (*Onobrychis sativa*) is a valuable fodder which provides high yields. If it is used in the green state until it starts flourishing, it does not cause bloating (Haţieganu et al., 1984).

Trefoil (*Lotus corniculatus*) is used for animal feed in the same quantities as the lucerne. It does not cause bloating. In certain climacteric areas, however, it may contains a toxic cyangenic glycoside (Haţieganu et al., 1984).

b) Annual legumes

There are two kinds of annual legumes: those which grow in spring and the others which grow in autumn.

Peas (*Pisum arvense*), is usually cultivated together with barley, oats, rye, and this mixture is known as mash.

Beans (*Vicia villosa, Vicia sativa, Vicia pannonica*) show antifungal and antibacterial activity, thanked to the phenolic compounds in the protein concentrate (Fernandez-Bats et al., 2018).

Lupine (*Lupinus luteus, L. albus, L. angustifolius*), contains a high protein content and low levels of anti-nutritional factors. Its use presents health benefits (Islam and Ma, 2016).

c) Annual grasses

Field corn (*Zea mays*), can be used as green fodder starting from June to late autumn. It is used once the plants have reached the height of 50-60 cm and

up to flowering. The nutritional value may vary within wide limits. It is poor in protein.

Rye (*Secale cereale*). It is gradually introduced in horses 'nutrition, mixed with fiber food (being administered up to 30 kg per day). It can be used when the plants are 20 cm height and up to flowering.

Oats (*Avena sativa*) Oats presents both advantages (very good tolerance and unsaturated fatty acids, mucilaginous substances and high digestibility) and disadvantages (the energy content is not sufficient for performance horses) (Pirkelmann et al., 2012).

Barley (*Hordeum vulgare*). It has a higher energy content than oats and hygienically speaking, it is less complicated than oats. But it also has some disadvantages: less unsaturated fatty acids (Pirkelmann et al., 2012).

Some perennial grass are also cultivated for the production of green fodder:

Fescue (*Festuca pratensis*), bluegrass (*Poa pratensis*), cocksfoot (*Dactylis glomerata*), and *Arrhenatherum elatius*. Their nutritional value increases when grown in combination with legumes. They are used in the form of grass, green fodder and especially as mowed green fodder (Haţieganu et al., 1984).

4.2. Coarse fibrous food

Coarse fibrous food is characterized by a high cellulose content and low nutritional value. This category includes straw, husks, cobs and herbs preserved by drying.

Cereal straw contains up to 50% crude fiber, 15-20% of the inlaid substances, and large amounts of silicic acid. Among these, oat straw is the most valuable, followed by wheat straw, spring barley, while winter cereal straw has a reduced digestibility (Haţieganu et al., 1984).

Hay, the basic food for herbivores during winter season offers up to 40% of the total energy and 50% of horses' required digestible protein. At the same time it represents a significant source of vitamins and nutritional substances. The nutritional value of hay depends on the floral composition, the age of the plants at harvest, the system used and the duration of drying and storage.

The optimal time of harvesting the crops for hay should be chosen to ensure a maximum quantity of hay and nutrients. This moment coincides with the phases of the grasses when they begin to flourish. Later harvesting of the plants may provide higher yields of hay, but lower quality with a high level of crude fiber, and significant loss of nutrients during drying.

Drying, storage and preservation of hay. There are two systems for drying: natural drying and artificial or technical drying (Haţieganu et al., 1984). The natural drying system is a widely applied method. In order to reduce the loss of plant nutrients, the drying is required to be performed in a short time. The duration of drying depends on the method used. These drying methods are in the furrow (Fig. 3), in waves, on surfaces, in the bundle.

Fig. 3. The drying system

4.3. Hay produced on natural grasslands

Natural hay characteristics are determined by natural climate, which classifies them into a particular type and sub-type (the type of hay is characterized by the natural conditions in which it was produced) (Haţieganu et al., 1984).

The natural area criteria provide several types and subtypes such as: swamp grass, meadow steppe, plain, hill, mountain, fen. Geographic variation affects the nutritional intake of equids (Mastellar et al., 2018).

Natural hay is used in nutrition of all animals. For cattle, it can be given in amounts of 5 to 12 kg / animal/day. Sheep, depending on age and physiological status, can receive between 0.5 and 2 kg / animal/day, and equines from 5 to 12 kg (Haţieganu et al., 1984).

The straw obtained from cultivated plants is made of perennial or annual grasses and legumes in pure culture or mixture. Lucerne is the most valuable. It has a high content of high biological value protein, minerals (especially Ca) and vitamins. The cellulose ratio should not be missing because cellulose influences favorably the intestinal transit in the cecum and colon by the growth of microorganisms. Reducing the intake of cellulose to 20-22% requires food richer in essential amino acids and vitamins (Marinier and Alexander, 1991; Hothersall and Nicol, 2009).

Horses cannot use the non-protein nitrogen in the synthesis of amino acids in the digestive tract. As a result, the biological value of the protein should be provided in the diet. The excess of protein increases the thermo production and the deterioration of nitrogen reserves of the body. Thus, the hay, preferably from natural grass, can meet the requirements of 40-80. The ratio may include:10-12% of barley and oat straw, succulent hay in 10-12%, 15-50% concentrated grains of oat (alone or mixed with other grains),10-20% wheat

bran, and 20-80% of green fodder (Murray et al., 2015; Sponheimer et al., 2003; Undersander, 2013).

Conclusions

Nutrition, a balanced diet and proper food management is vital in equines' care. Performance horses need a lot of muscle mass, being fed with large amounts of grain concentrate, and the program is strict including meals three times a day. Not only the body mass but also the physical performance, the competitiveness and the image of the horse are considered a reflection of the energy intake including the intake of proteins, nutrients and vitamins. The nutritional value and the production of the hay are determined by the association of grassland flora and vegetation. In terms of nutrition, natural plants on the fields are grouped into: grasses with high nutritional value, average nutritional value and low nutritional value.

Rezumat. Hrănirea şi nutriţia calului instruit pentru performanţă este mult mai complexă decât hrănirea altor categorii specializate. Acest lucru se datorează stresului, efortului la care acesta este supus. Din păcate, încă este un subiect incomplet, având puţine informaţii cu privire la cerinţele nutritive optime ale calului. Trebuie să folosim informaţiile obţinute de la animale şi cu sportivul uman, precum şi experienţa călăreţilor de vârf, pentru a ajuta la formularea programelor de hrănire şi nutriţie pentru această categorie de cal. Acestea pot fi utilizate până la obţinerea unor informaţii cu adevărat ştiinţifice care pot îmbunătăţi programul urmărit. Într-un sens, calul de curse este un sportiv a cărui cerinţe nutriţionale sunt foarte ridicate şi exigente, dar puţin cunoscute. Prin urmare, nu este surprinzător faptul că doar un mic procent de cai de rasă care au început să se antreneze rămân sănătoşi după un an competiţional. Această lucrare descrie cerinţele nutritive la caii de performanţă, interrelaţia dintre aportul de plante şi modul de selectare a lor în funcţie de diferite criterii, valoarea nutritivă oferită de către plante oglindindu-se în preferinţele acestuia.

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SPATIALLY DISTRIBUTION OF SMALL MAMMALS FROM RODNA MOUNTAINS NATIONAL PARK, ROMANIA

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Abstract. Between 2004-2017 a study of the small mammals of the natural ecosystems from Rodna Mountains National Park (Eastern Carpathian Mountains) revealed the identification of 23 species among 924 individuals captured in 10 habitats along 500 transects by using live-traps for small mammals (Sherman type). The altitude of the investigated habitats was between 600-1.600 m, from mountain valleys to glacial circuses.

Some small mammals as *Sicista betulina, Neomys anomalus* are rare species and others like *Apodemus sylvaticus, Apodemus flavicollis, Clethrionomys glareolus* are common. Among the identified species, some of them (*Microtus tatricus, Sicista betulina, Muscardinus avellanarius, Dryomys nitedula, Sorex alpinus, Neomys anomalus*) are considered protected species in Romania (49/2011 Law). From 23 inventoried species, 16 species are rodent and 7 species are insectivorous.

Key words: small mammals, protected, rare.

Introduction

Rodna Mountains National Park is the second national park in our country, with an area of 47.000 ha. The importance of this protected area is due both to the geology and geomorphology of the mountains and to the presence of many species of fauna and flora, endemites and glacial relics. The Rodna Mountains, which show the highest altitudes in the Eastern Carpathians (Pietrosu Rodnei Peak – 2,303 m, Inău Peak – 2,279 m), are located in the northern part of Romania. Being included in the Nordic group, also known as the Carpathians of Maramureş and Bucovina, these mountains dominate the landscape, with the largest differences in the level being recorded against the Maramureş Depression, located to the north. From an administrative point of view, the Park extends in the counties of Maramureş and Bistrita-Nasaud. The Rodna Mountains National Park does not include the entire chain of the Rodna Mountains but only a part of them. From the West to the East, along a main ridge of about 55 km (from Şetref to the Rotunda), and from North to South, on a 25 km length, from Prislop Pass to Valea Vinului – Bistriţa-Năsăud county (www.parcrodna.ro, fig. 1).

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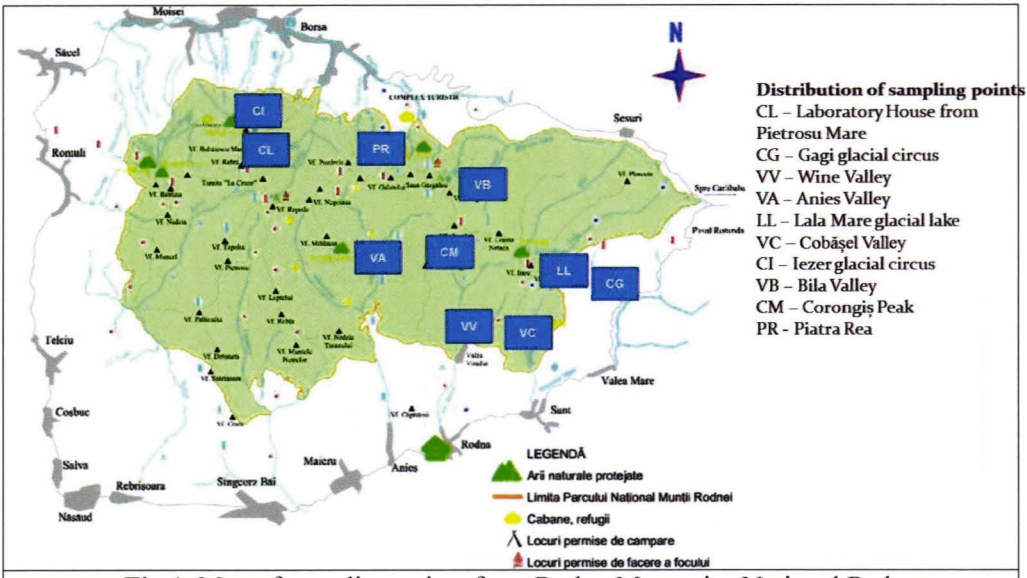

Fig.1. Map of sampling points from Rodna Mountains National Park

The small mammals fauna inventory study broke out during the period 2004-2017 and also involved an analysis of the bibliography on the research conducted so far. Bats species were not included because these aspects were included in a similar study.

The term "small mammals" means mammals with a body weight less than 1 kg of Rodentia, Insectivora and Chiroptera. The analysis of the wealth of species in relation to the mass reveals that over 75% of the current mammals fall into the group of micromamifers, being the most common species in any mammalian community (Entwistle&Stephenson, 2000).

The importance of small mammals within ecosystems mainly relates to their role in trophic networks both as a trophic base and as predators (MacDonald&Barret, 1993), to direct influence on the structure and composition of vegetal communities through consumption and seed dispersal (Andersen et al., 2000) and on invertebrate communities (Churchfield, 1990).

From a conservative point of view, the level of threat of disappearance is as large among small mammals as in large mammals, but large species are those thatare almost exclusively protected by protection programs, although most of the species that have disappeared in recent times or are likely to become extinct over the next 30 years are part of the small mammals category (Entwistle&Stephenson, 2000).

Threats to small mammals are (as in the case of large mammals) habitat loss, pollution, introduced species, hunting and persecution, but they are more exposed to the degradation and destruction of micro-habitat conditions (Entwistle&Stephenson, 2000). The loss and degradation of habitats is the

biggest delay for small mammals (IUCN, 2008), especially for those strictly associated with forest habitats (Entwistle&Stephenson, 2000).

The first data on the fauna of the Maramures region belongs to Hanak (1848 ap. Szilágyi 1876), which describes the observation of the *Marmota marmota* in the Maramures Mountains as well as its whistling in Pietrosu Rodnei. The first faunistic list for Maramures, including also mammals, was elaborated by Frivaldsky (1875). In 1960, Szabó published a paper on small mammals from the northwestern part of Romania, including data from the Rodna Mountains. More recently, some papers were published, including data on the small mammal species from different regions of the Maramureş region: Ardelean&Bereş (2000) - Vaser Valley, Murariu and Răduleţ (1998) - Maramureş Depression.

The description of the habitat where a specimen of *Sicista betulina* was captured from the face of Pietrosu Rodnei, together with a synthesis on current knowledge of this species in Romania, is presented by Murariu (1995, 1997). In a paper concerning the genus *Arvicola* in Romania (Murariu, 2000) information from Maramureş Depression is also presented. Wagner (1974) published a PhD thesis on small mammals from the Inner Carpathian Basin, including data from Rodna Mountains. However, as there are no details concerning the sampling points, it is possible that at least some of the data come from the southern part of Rodna Mountains, outside the Tisa River Basin. In 2000, Ardelean and Béres published a synthesis on the vertebrate's fauna from Maramureş. In volume dedicated to the Maramureş Mountains Nature Park of the "Transylvanian Review of Systematically and Ecological Research" (Sibiu, 2008), Gurzău et al. published a paper on small mammals. In the same journal, in the volume dedicated to Rodna Mountains National Park (2008) two other papers were published on small mammal's fauna (Nae et al., 2010) and on the present distribution of *Marmota marmota* (Szabo, 2010). Data concerning the distribution of the population of reintroduced marmots from Rodna Mountains were previously published (Almăşan 1981; Pânzariu 1993; Nădişan 2000).

Materials and methods

In the case of the small mammals of the Rodentia and Insectivora orders, population estimation is an extremely difficult process for which individuals are tagged, which in most cases involves catching animals, so the use of traps is the most widespread method used in the research of micromamps. The traps used are live-trap, with which micromamps are captured alive.

The capture of live animals is necessary due to the protective status of some species and also to avoid changing the structure of the populations by sacrificing the captured individuals. Pitfalls are wooden boxes (18x7,5x9 cm) provided with a pedal which, when touched by the animal, causes the fall and blocking of the sheet door so that the animal remains trapped inside.

The bait used was made of roasted sunflower and pumpkin seeds, walnuts and bacon. 300 traps were used. The captured animals were anesthetized with chloroform.The live-traps were placed in linear transects. The distance between two successive traps was 15 m, a model that complies with the rule that the distance between two successive traps is not greater than the radius of a circle having the area equal to the surface area of the target species (Jones et al., 1996).

For small mammal diversity studies, the smallest area is taken into account, resulting in the placement of a trap in each individual territory. The duration of the pitfalls was determined by two considerations: in order to be effective, traps must operate in such a way as to capture at least 3 activity periods of the target species. But at the same time they do not have to be active for too long to limit variations in population. It has been assumed that during the three-day study, the population is closed, respectively, mortality, immigration and emigration are negligible (Jones et al., 1996). In the period 2004-2017 a series of inventories of fauna of small mammals were made using catching-tagging-releasing-recapturing method. Box-type wooden traps with food were used to attract and survive animals.

Results and discussions

The conservative value of an area is determined by estimating the diversity of taxonomic groups (Sutherland et al., 2000), a process that begins by compiling faunistic lists and estimating specific wealth (May, 1988; Rudran&Foster, 1996). The richness of species in a certain area can also be used as a measure of determining the priority areas for conservation (Myers et al., 2000), being even a more user-friendly indicator of community dynamics than estimates of abundance, which can vary strongly, in an irregular manner to the micro-mills in temperate Europe (Hansson&Henttonen, 1985).

In order to effectively protect and conserve these species, it is important to know the factors that limit their distribution and shape the structure of the dormice communities (Bright et al., 1996). Habitat selection by each species is considered a major factor in community dynamics (MacArthur&Levins, 1964, Benedek, 2008), and the distribution and abundance of species is determined by differences in resource availability and habitat quality as a function of intrinsic habitat factors and of the density of the competitors (Guthrie&Moorhead, 2002, Popescu&Murariu, 2001).

Nesting sites may be a limited resource for small mammals (Dooley&Dueser, 1990; Bright&Morris, 1996), especially for nesting species in cavities. The majority of dormice species prefer to nest in the trees (Pucek, 1981; Görner&Hackethal 1988; Juškaitis, 2003; Bright et al., 1996; Torre, 2004), which satisfy their thermoregulation and protection requirements against

predators (Bright&Morris, 1996). These species do not excavate their own scabs, but they deal with the secondary ones (secondary cavity nesters).

Species nesting in wooden cavities form a community that interacts and competes for nesting places (Barba&Gil-Delgado, 1990; Aitken et al., 2002; Koppmann-Rumpf et al., 2003). For species with naturally occurring or active species digesting (such as Gliridae species), their availability may be a limiting factor (Pöysä&Pöysä, 2002; Juškaitis, 2003).

We identified 23 species of small mammals in 10 locations in the Rodna Mountains National Park. 500 transects were applied, respectively 5 transects in each location between 2004 and 2017 (Table 1).

Table 1. Species and number of individuals of small mammals identified in the Rodna Mountains National Park during 2004-2017

No.	Species	Location and number of individuals recorded										No. of individuals
		CL	CG	VV	VA	LL	VC	CI	VB	CM	PR	
1	Apodemus sylvaticus	57	7	76	16		59		5	3	2	225
2	Apodemus flavicollis	43	3	68	12		46		4	2		178
3	Apodemus agrarius			19	5							24
4	Microtus agrestis			18	3	9					3	33
5	Microtus arvalis	8	8	8	6	5					1	36
6	Microtus nivalis		18			17		18	6	7	4	70
7	Microtus tatricus		8			7				15	6	36
8	Cletrhionomys glareolus	36		47	2		33					118
9	Sorex araneus	3					6					9
10	Sorex alpinus	14		12	4	5	9	6	3	1	2	56
11	Sorex minutus			8	1		3					12
12	Neomys fodiens			2			2		4	1		9
13	Neomys anomalus				2							2
14	Crocidura leucodon			6	3		4		1			14
15	Talpa europaea	1		2	1		2					6
16	Pitymys subterraneus	2	2				5	2	2	1	1	15

No.	Species	Location and number of individuals recorded										No. of individuals
		CL	CG	VV	VA	LL	VC	CI	VB	CM	PR	
17	Muscardinus avellanarius	6	4	8	4	3	11	5	1	2	1	45
18	Dryomys nitedula			4								4
19	Myoxus glis	3	1	3			4				1	12
20	Sicista betulina							1				1
21	Sciurus vulgaris	2		3			3					8
22	Arvicola terrestris			1			2					3
23	Mus musculus	3		2			1		2			8
Total number of individuals		**12**	**8**	**19**	**12**	**6**	**16**	**5**	**9**	**8**	**9**	**924**

The code of location for each transect: CL – Laboratory House from Pietrosu Mare (1.400 m, coniferous forest, mixed forest); CG –Gagi glacial circus (1.500 m, pasture with bush, *Juniperus, Rhododendron*); VV – Wine Valley (800 m, ecoton of mixed forest, mountain valley); VA – Anies Valley (1.200 m, limestone rocks, pasture, mixed forest, coniferous forest); LL –Lala Mare glacial lake (1.600 m, pasture with bush, *Juniperus, Rhododendron*); VC –Cobăşel Valley (900 m, mountain valley, mixed forest); CI –Iezer glacial circus (1.700 m, pasture with bush, *Juniperus, Rhododendron*); VB –Bila Valley (1.200 m, higrophilous meadow, pasture); CM – Corongiş Peak (1.500 m, limestone rocks, pasture); PR - Piatra Rea (1.300 m, limestone rocks, pasture).

The common and rare species from each investigated habitat is showed in the figures 2-11.

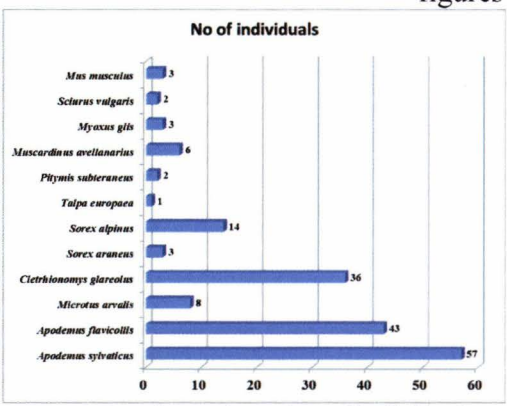

Fig. 2. Species and number of individuals captured at Laboratory House

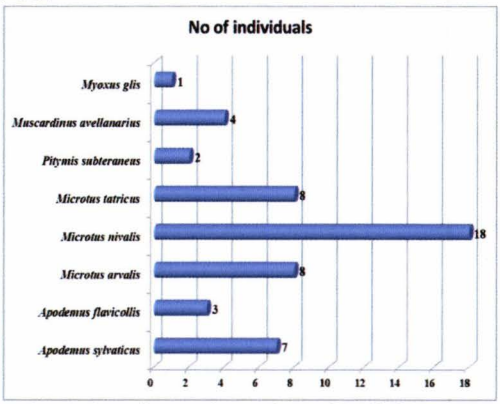

Fig. 3. Species and number of individuals captured from Gagi glacial circus

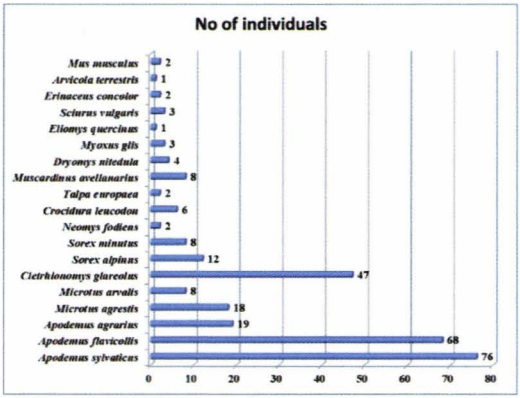

Fig. 4. Species and number of individuals captured from Wine Valley

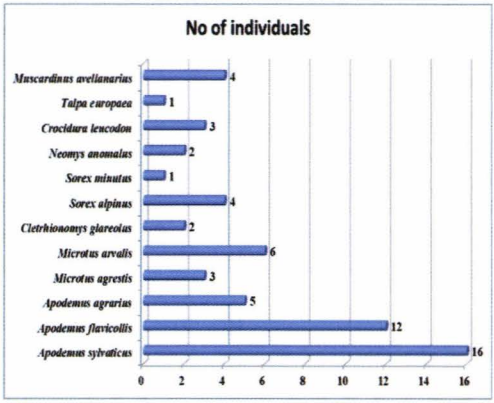

Fig. 5. Species and number of individuals captured from Anies Valley

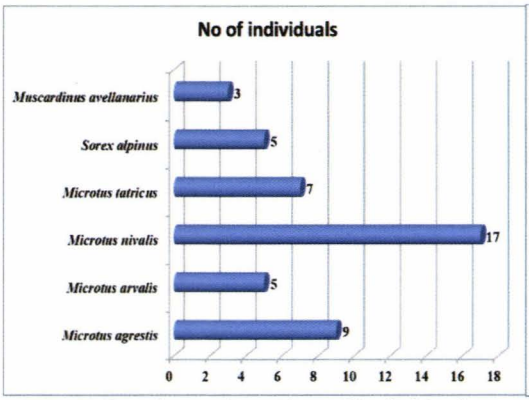

Fig. 6. Species and number of individuals captured from Lala Mare glacial lake

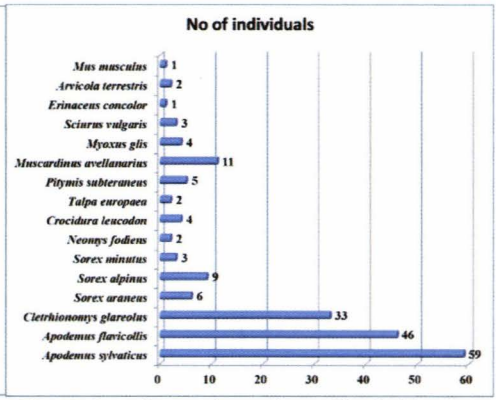

Fig. 7. Species and number of individuals captured from Cobasel Valley

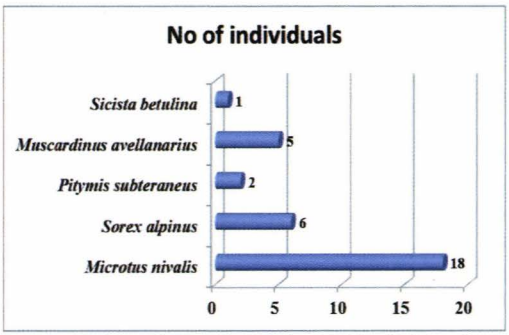

Fig. 8. Species and number of individuals captured from Iezer glacial circus

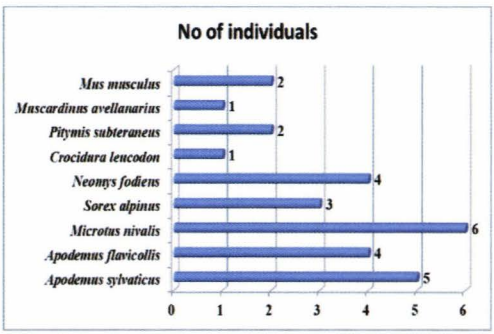

Fig. 9. Species and number of individuals captured from Bila Valley

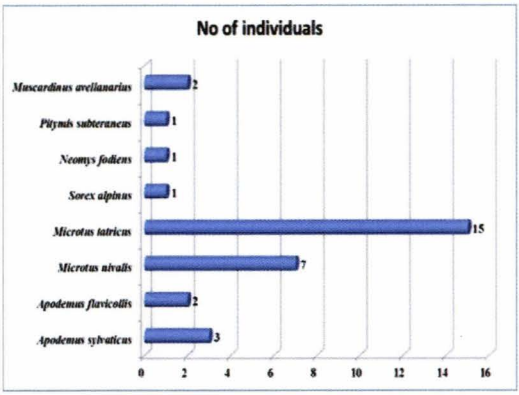

Fig. 10. Species and number of individuals captured from Corongis Peak

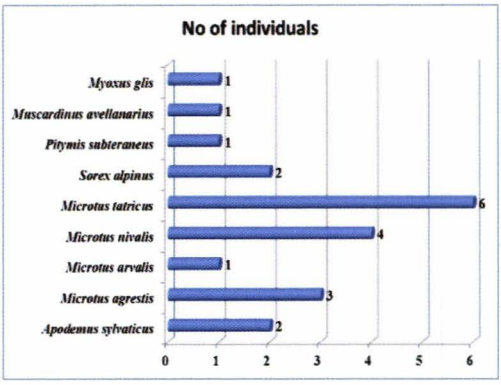

Fig. 11. Species and number of individuals captured from Piatra Rea

As can be seen in the fig. 2, the most common species in the area of Laboratory House from Pietrosu Mare are *Apodemus sylvaticus, A. flavicollis, Clethrionomis glareolus*, being the same situation like at Wine Valley (fig.4). In the case of Gagi glacial circus (fig. 3) the common species are *Microtus nivalis, M/. tatricus, M. arvalis*. A vey interesting capture at Iezer glacial circus was the *Sicista betulina* (Northern birch mouse), a very rare species in Rodna Mountains, being captured also by Murariu in 1996 and published in 1997. Other important and protected species is *Microtus tatricus*, captured in Piatra Rea, Corongis Peak, Lala Mare lake, Gagi glacial circus (fig. 10, 11).

From species richness point of view, the high number of species were identified in Wine Valley, Cobasel Valley, Anies Valley, Laboratory House from Pietrosu Mare because there is a complex mozaic of habitats (fig. 12).

Taking into account the number of individuals for all investigated sites, according to figure 13, can be seen that some species were captured in a high number, being comon species (*Apodemus sylvaticus, A. flavicollis, Cletrhionomys glareolus*) and some of them are very rare (*Sicista betulina, Neomys anomalus*).

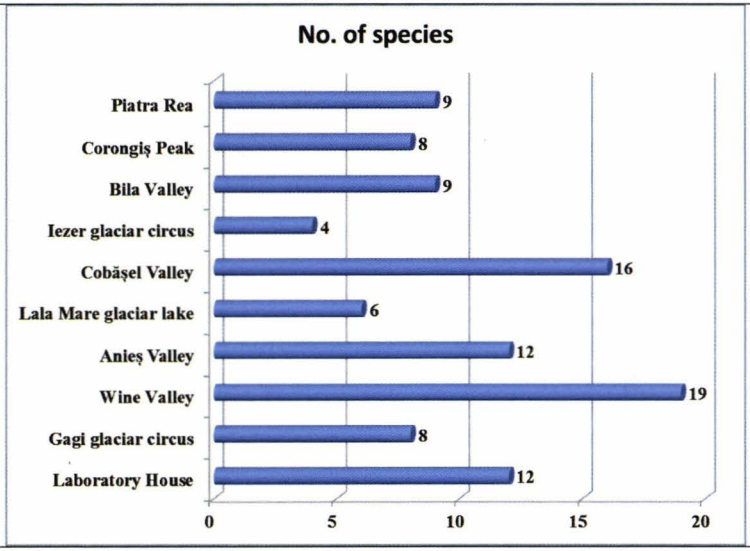

Fig. 12. Species richness of investigated habitats from Rodna Mountains National Park

By analysing the trophic regim, there are many rodents than insectiovres (fig. 13).

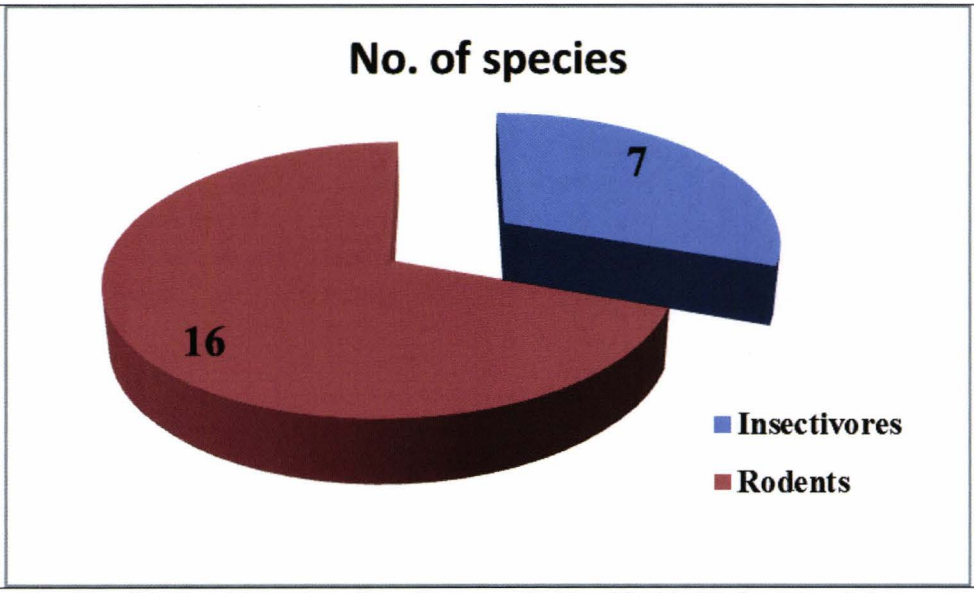

Fig. 13. Trophic regim of small mammals identified in Rodna Mountains National Park

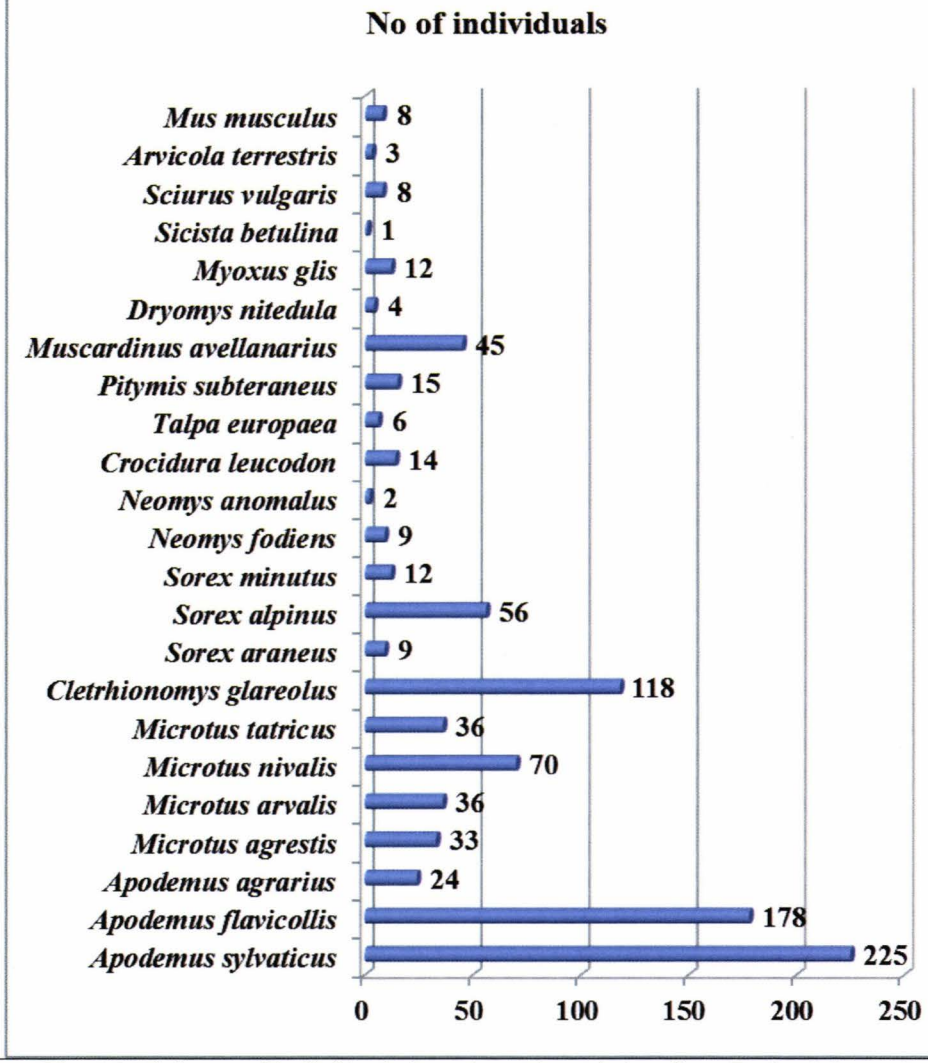

Fig. 14. Richness of individuals from investigated sites from Rodna Mountains National Park

Conclusions

During the period 2004-2017, we conducted a study of small mammals from nature ecosystems of Rodna Mountains National Park (Eastern Carpathians) and there were identified 23 species and 924 individuals captured in 10 sites along 500 transects by using live-traps (Sherman type). The altitude of investigated sites were between 600-1.600 m, from mountain valleys up to glacial circuses.

Some species of small mammals are very rare (*Sicista betulina, Neomys anomalus*) and other are very common (*Apodemus sylvaticus, Apodemus*

flavicollis, Clethrionomys glareolus). From the identified species, some of them are protected in Romania (Law 49/2011) such as: *Microtus tatricus, Sicista betulina, Muscardinus avellanarius, Dryomys nitedula, Sorex alpinus, Neomys anomalus*. From 23 species, 16 species are rodents and 7 species are insectivores.

Rezumat. În perioada 2004-2017 s-a desfăşurat un studiu al mamiferelor mici din ecosistemele naturale ale Parcului Naţional Munţii Rodnei (Carpaţii Orientali) şi au fost identificate 23 de specii şi 924 de indivizi capturaţi în 10 habitate de-a lungul a 500 de transecte. folosindu-se capcane ce capturează animale vii (tip Sherman). Altitudinea habitatelor investigate a fost între 600-1.600 m, de la văi de munte până la circuri glaciare.

Unele specii de micromamifere sunt rare la nivelul masivului (*Sicista betulina, Neomys anomalus*) şi altele sunt frecvente (*Apodemus sylvaticus, Apodemus flavicollis, Clethrionomys glareolus*). Dintre speciile identificate, unele sunt protejate în România (Legea 49/2011) cum ar fi: Mic*rotus tatricus, Sicista betulina, Muscardinus avellanarius, Dryomys nitedula, Sorex alpinus, Neomys anomalus*. Din cele 23 de specii inventariate, 16 specii sunt rozătoare şi 7 specii sunt insectivore.

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CONSIDERATIONS ON INTESTINAL INFECTION DUE TO *E. COLI*

Liana Monica DEAC*

Abstract. *Escherichia coli* is a type of bacteria that normally lives in the intestines of people and animals. However, some particular strains, as *E. coli 0157:H7*, can cause intestinal infection. Symptoms of such intestinal infection include: diarrhea, abdominal pain and fever. More severe cases can lead to bloody diarrhea, dehydration, or even kidney failure. People with weakened immune systems, pregnant women, young children and older adults are at increased risk for developing this infection. Infection can occur in people of all ages, but is most common in children. Certain toxic strains may lead to serious illness, because they produce a toxin called *Shiga*, or *Verocytotoxin*, which causes severe illness and bleeding, that can be fatal, especially in children. *Shiga-producing toxic E. coli,* often called "*STEC*" for short, may be referred to as *E. coli 0157*. Such toxin can cause severe illness, with bloody diarrhea and hemolytic uremic syndrome (*HUS*), which is a type of kidney failure. Remarkable is that most intestinal infections are caused by contaminated food or water, or by infected people. Proper food preparation, correct hand washing and good hygiene can greatly decrease the developing an intestinal infection. Usual cases of intestinal *Escherichia coli* infection can be treated at home and symptoms generally resolve within a few days to a week. Severe cases must be hospitalized, being life-threatening.

Key words: *Escherichia coli*, *Shiga toxin*, intestinal infection.

Introduction

Enteritis is an inflammation or swelling of the intestines, with bacterial causes (CDC, 2009). People usually get enteritis caused by *E. coli* from eating raw or uncooked foods, drinking untreated water and unsafe food handling. Most *E. coli*, in fact, hundreds of strains, are harmless and actually are an important part of a healthy human intestinal tract. However, certain toxic strains may lead to serious illness. Some strains are more dangerous than others, because they produce a toxin called *Shiga*, or *Verocytotoxin* (Boyce et al., 1995). This toxin causes severe illness and bleeding that can be fatal, especially in children. *Shiga-producing toxic E. coli,* often called "*STEC*" for short, may also be referred to as *E. coli 0157*. According to the Center for Disease Control and Prevention (CDC, 2009), many estimated *STEC infections* occur each year in whole world. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons.

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In rare cases, a severe infection can cause hemolytic uremia, which can lead to

Considerations on *Escherichia coli* strains

E. coli consists of a diverse group of bacteria. Pathogenic *E. coli* strains are categorized into path types. Six path types are associated with diarrhea and collectively are referred to as diarrheagenic *E. coli*: Shiga toxin-producing *E. coli* (STEC)-STEC may also be referred to as Verocytotoxin-producing *E. coli* (VTEC) or entero- hemorrhagic *E. coli* (EHEC). This path type is the one most commonly heard about in the news in association with food borne outbreaks (Griffin and Tauxe, 1991). There are also known today: Enter toxigenic E. coli (ETEC), Enter pathogenic *E. coli* (EPEC), Enter aggregative *E. coli* (EAEC), Enter invasive *E. coli* (EIEC), Diffusely adherent *E. coli* (DAEC), with certain pathology. The strain of Shiga toxin-producing *E. coli* O104:H4 that caused a large outbreak in Europe in 2011 was frequently referred to as EHEC. The most commonly identified STEC in North America is *E. coli* O157:H7 (often shortened to *E. coli* O157 or even just "O157"). In addition to *E. coli* O157, many other kinds (called serogroups) of STEC cause disease. Other *E. coli* serogroups in the STEC group, including *E. coli* O145, are sometimes called "non-O157 STECs."

Currently, there are limited public health surveillance data on the occurrence of non-O157 STECs, including STEC O145; many STEC O145 infections may go undiagnosed or unreported. Compared with STEC O157 infections, identification of non-O157 STEC infections is more complex. First, clinical laboratories must test stool samples for the presence of Shiga toxins (Johannes, 2010).

Receptivity and risk for *Escherichia coli* enteritis

People of any age can become infected. Almost everyone has some risk of infection. Very young children and the elderly are more likely to develop severe illness and hemolytic uremic syndrome (HUS) than others, but even healthy older children and young adults can become seriously ill. STEC live in the guts of ruminant animals, including cattle, goats, sheep, deer, and elk. The major source for human illnesses is cattle. STEC that cause human illness generally do not make animals sick. Other kinds of animals, including pigs and birds, sometimes pick up STEC from the environment and may spread it (Rangel et al., 2005).

Source, exposure, transmission and risk for the infection

Exposures that result in illness include consumption of contaminated food, consumption of unpasteurized (raw) milk, consumption of water that has not been disinfected, contact with cattle, or contact with the feces of infected

people. *E. coli* O157:H7 bacteria and other pathogenic *E. coli* mostly live in the intestines of cattle, but *E. coli* bacteria have also been found in the intestines of chickens, deer, sheep, and pigs. Therefore, eating not well prepared meat or food can be a big risk for the infection outbreak. Beef and dairy cattle are known reservoirs of *E. coli* O157:H7 and non-O157 Shiga toxin-producing strains of *E. coli*. Some other foods are considered to carry *E. coli* O157, as unpasteurized raw milk, unpasteurized apple cider, and soft cheeses. Sometimes the contact is pretty obvious by working with cows or eating an undercooked hamburger or a contaminated piece of lettuce. People have got infected by swallowing lake water while swimming, touching the environment in petting zoos and other animal exhibits, and by eating food prepared by people who did not wash their hands well after using the toilet. Young children tend to carry STEC longer than adults (Robins- Browne, 2005). A few people keep shedding these bacteria for several months. People at higher risk for food borne illness are pregnant women, newborns, children, older adults, and those with weak immune systems.

Clinical aspect of the infection and its complication

The symptoms vary for each person but often include severe stomach cramps, diarrhea (often bloody), and vomiting. If there is fever, it is usually not very high (less than 101°F/less than 38.5°C). Most people get better within 5–7 days. Some infections are very mild, but others are severe or even life-threatening (Welinder-Olsson and Kaijser, 2005). The incubation period is usually 3-4 days after the exposure, but may be as short as 1 day or as long as 10 days. The symptoms often begin slowly with mild belly pain or non-bloody diarrhea that worsens over several days. Not surprisingly, *E. coli* O157:H7 infection is associated with long-term emotional disruption as well, not just for the victim, but for entire families. HUS, if it occurs, represents a complication and develops on average 7 days after the first symptoms, when the diarrhea is improving (Wong et al., 2000). It accounts for the majority of the acute deaths and chronic injuries caused by the bacteria. By definition, when fully expressed, HUS presents with the triad of hemolytic anemia (destruction of red blood cells), thrombocytopenia (low platelet count), and renal failure (loss of kidney function). The duration of an uncomplicated illness can range from one to twelve days.

Laboratory diagnosis

Most hospitals labs and physicians know to test for these particular bacteria, especially if the potentially infected person has bloody diarrhea. Patients with more severe illness are generally more likely to seek medical care and to have stool specimens tested to determine the cause of illness. Clinical laboratories should report and send *E. coli* O157 isolates and Shiga toxin-

positive samples to state or local public health laboratories as soon as possible for additional characterization. Many infected people do not seek medical care; many of those who do seek care do not provide a stool specimen for testing, and many labs do not test for non-O157 STEC. However, this situation is changing as more labs have begun using newer, simpler tests that can help detect non-O157 STEC. STEC infections are usually diagnosed through laboratory testing of stool specimens (feces). There are two general types of Shiga toxins, Shiga toxin 1 and Shiga toxin 2. Strains may carry one Shiga toxin or both at the same time. Strains that produce Shiga toxin 2 tend to be more virulent. Many labs can determine if STEC are present, and most can identify *E. coli* O157. Labs that test for the presence of Shiga toxins in stool can detect non-O157 STEC infections (Boyce et al., 1995).

Treatment

Supportive therapy, including hydration, is very important. Taking antibiotics may increase the risk of HUS and therefore should not be used to treat this infection. Anti diarrheal agents may also increase that risk (Rangel et al., 2005). Most people get better within 5–7 days. Some infections are very mild, but others are severe or even life-threatening. Persons with HUS should be hospitalized because their kidneys may stop working and they may develop other serious problems. Most persons with HUS recover within a few weeks, but some suffer permanent damage or die.

Prevention measures and infection control

Good hand-washing is always a smart idea to protect yourself, your family, and other persons. Wash your hands thoroughly after using the bathroom, changing diapers, and before preparing or eating food. Wash your hands thoroughly after contact with animals or their environments. Alcohol-based products can quickly reduce the number of germs on hands in some situations, but they are not a substitute for washing with soap and running water. Practice proper hygiene and keep all objects that enter infants' and toddlers' mouths clean. Cook meats thoroughly. Avoid drinking and eating unpasteurized milk or other unprepared hygienic products. Prevent cross-contamination in food preparation areas by thoroughly washing hands, counters, cutting boards, and utensils after they touch raw meat. Avoid drinking any non-chlorinated water or swallowing water when swimming. All patients with Shiga toxin-positive diarrheal illness or HUS should be reported to health departments (CDC, 2009).

Conclusions

1. Most *E. coli* are harmless, but certain toxic strains, as virulent *STEC*, are and may lead to serious illness, when the infection can occur in people of all ages but is most common in children.

2. The colitis caused by *Escherichia coli* O157:H7 is characterized by severe abdominal cramps, diarrhea that typically turns bloody within 24 hours, and sometimes exhibits fever. Some cases can recover within a few weeks while some subjects suffer permanent damage, or even die.

3. Infection with *E. coli O157:H7,* or other *Shiga toxin-producing E. coli*, is usually confirmed by the detection of the bacteria in a stool specimen from an infected individual, but many infections continue to go undiagnosed today.

4. Identifying the specific strain of *STEC* is essential for public health purposes, such as finding possible outbreaks.

5. Good hand-washing before preparing and eating food, or after contact with animals and their environments, proper hygiene, are the best prevention measures of the infection spread.

Rezumat. *Escherichia coli* este o bacterie care trăieşte în intestinul oamenilor şi al animalelor. Totuşi unele tipuri particulare, ca *E. coli 0157:H7,* pot cauza infecţii intestinale. Simptomele acestora includ: diaree, colici şi febră. Cazurile severe pot prezenta diareee hemoragică, deshidratare, sau chiar insuficenţă renală. Persoanele cu deficienţe ale sistemului imun, gravidele, copiii mici, vîrstnicii, se află la risc mare de a face asemenea stări infecţioase. Infecţia poate fi prezentă la orice vîrstă, dar este mai des întâlnită la copii. Anumite tulpini toxice pot duce la manifestări severe de boală, pentru că produc *toxina Shiga.*sau *Veroxitoxina*, care declanşează îmbolnăviri severe şi hemoragii care pot fi fatale, mai ales printre copii. *E. coli* producătoare de toxina *Shiga*, numită scurt "*STEC*", se referă la *E. coli 0157*. Toxina cauzează boli grave, cu diaree hemoragică, sindrom hemoragic uremic (*SHU*), insuficienţă renală tipică. De remarcat este faptul că majoritatea acestor infecţii intestinale sunt cauzate de contaminarea mîncării sau a apei de băut, sau provin de la bolnavii efectiv infectaţi bacterial. Mâncarea bine preparată termic, spălatul corect al mâinilor şi igiena bună individuală, pot scădea sugestiv apariţia acestor infecţii intestinale. Cazurile uzuale de îmbolnăvire pot fi tratate şi acasă, iar simptomele cedează în câteva zile până la o săptămână. Cazurile severe se internează, însă, pentru a li se putea salva viaţa.

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