Consiliul Județean Bistrița-Năsăud

Complexul Muzeal Bistrița-Năsăud

COMPLEXUL MUZEAL
BISTRIȚA NĂSĂUD

# STUDII ŞI CERCETĂRI
## Biology

23-24

BISTRIȚA

2019

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BISTRIŢA-NĂSĂUD

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EDITORIAL BOARD OF Series Biology
Published by the Museum of Bistriţa-Năsăud, Natural Sciences Section
Editorial office: 19 Gen. Grigore Bălan St.
420016 BISTRIŢA
Phone: 004 0263 211063
Fax: 0263 230046



Revista „Studii şi cercetări" seria Biology, numerele 23-24, a fost tipărită în anul 2021, apariţia simultană a celor două numere fiind determinată de situaţia specifică Pandemiei de COVID-19.



**Editura ECOU TRANSILVAN**
**ISSN 2069 - 1521**

## CUPRINS. CONTENT. SOMMAIRE. INHALT

# *LAUDATIO* – PROF. UNIV. EMERIT DR. MIHAIL DRĂGAN BULARDA CU OCAZIA ANIVERSĂRII A 80 DE ANI

Rahela CARPA*, Marius HORGA**

Profesorul univ. emerit dr. **Mihail Drăgan Bularda** s-a născut la data de 10 octombrie 1938 în localitatea Cooc (actualmente Pădureni), com. Tritenii de Jos, jud. Cluj. A absolvit Colegiul Naţional Mihai Viteazu din Turda, apoi Universitatea „Babeş-Bolyai" din Cluj-Napoca, Facultatea de Biologie şi Geografie, secţia Biologie-Botanică.

Debutul în cariera universitară a fost iniţial ca preparator prin repartiţie ministerială, în 1963, parcurgând apoi toate treptele universitare. Totodată, domnul profesor a urmat specializări în ţară la Universitatea Bucureşti, la Acad. Gh. Zarnea şi la Facultatea de Medicină Veterinară Bucureşti, prof. Nicolae Stamatin, iar în străinătate la Universitatea din Tübingen, RFG, respectiv la Institutul de Pedologie şi Agrochimie, Laboratorul de Microbiologie din München, RFG.

Şi-a susţinut doctoratul cu o teză din domeniul Microbiologiei şi Enzimologiei solului – *Studii asupra unor polizaharidaze şi transferaze din sol,* teză coordonată de academicianul profesor dr. Ştefan Peterfi.

Cariera de cadru didactic a cuprins lucrări practice şi cursuri predate la studenţi: Microbiologie generală, Microbiologia solului, Biotehnologii microbiene, Microbiologia mediului. De asemenea, a mai predat cursuri şi lucrări practice de Microbiologie şi la Facultatea de Ecologie a Universităţii „Lucian Blaga" din Sibiu, precum şi la Universitatea din Oradea. De asemenea, a coordonat zeci de lucrări de licenţe şi disertaţii ale studenţilor.

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O altă latură a activității didactice a fost aceea de îndrumare a profesorilor de Biologie din Învățământul secundar (liceal sau general).

În acest sens a efectuat inspecții speciale pentru acordarea gradelor didactice II și I, îndrumând peste 50 lucrări metodico-științifice în vederea obținerii gradului didactic I. S-a remarcat și prin participarea ca referent științific în comisii de doctorat, la mai multe universități din țară ca Universitatea „Babeș-Bolyai", Universitatea „Iuliu Hațieganu", Universitatea de Științe Agricole și Medicină Veterinară din Cluj-Napoca, Universitatea „Al. Ioan Cuza" Iași, Universitatea de Științe Agricole din Timișoara, Universitatea „Ovidiu" din Constanța.

Cariera de cercetător cuprinde peste 300 lucrări științifice publicate în reviste de specialitate din țară, respectiv 30 în reviste din străinătate. De asemenea, a publicat 6 manuale universitare destinate studenților și nu numai. Este coautor la 4 cărți apărute, 3 în țară, iar una în străinătate: *Enzymology of Disturbed Soils,* la Ed. Elsevier din Amsterdam, în anul 1998.

A participat la foarte multe manifestări științifice în țară și la 5 manifestări științifice în străinătate cu prezentări orale sau prezentări de postere. A activat ca secretar de redacție la revista *Studia* a Universității „Babeș-Bolyai", seria „Biologia" și apoi ca președinte timp de câțiva ani. Este membru în board-ul editorial și referent științific al revistei *Studii și Cercetări* seria „Biology" a Complexului Muzeal Bistrița-Năsăud. De asemenea, a făcut parte din Societatea Română de Știința Solului, activând câțiva ani ca președinte la secția de Biologia solului. A mai activat și în cadrul Societății de Științe Biologice din România. Face parte și din colectivul redacțional al revistei *Analele Universității din Oradea*, Fascicula Biologia.

În anul 1993 a primit **premiul Academiei Române „Em. C. Teodorescu"** pentru lucrarea în colaborare *Enzimologia mediului înconjurător* apărută la Ed. Ceres, București. A făcut parte dintr-un colectiv de cercetare al Universității „Babeș-Bolyai", condus de prof. univ. dr. Stefan Kiss, în colaborare cu Institutul de Cercetări Biologice Cluj, care a studiat peste 20 de ani, pe baza contractuală, și în colaborare cu Institutul de Balneologie și Medicină Fizică din București, respectiv cu Institutul Geologic București, lacurile saline din România din următoarele stațiuni balneare: Turda, Cojocna, Someșeni, jud. Cluj, Sovata, Ocna Sibiului, Ocna Șugatag și Coștiui din județul Maramureș, Slănic Prahova, Lacu Sărat – Brăila, Costinești, Techirghiol, Nuntași.

În calitate de conducător de doctorat în domeniul Microbiologiei, a condus 20 lucrări de doctorat, dintre care 18 au obținut titlul de doctor în biologie.

În anul 2011 a primit titlul onorific de profesor emerit, pe baza criteriilor cerute de Ministerul Educației, din partea Universității „Babeș-Bolyai", Cluj-Napoca.

Pentru toate cele amintite mai sus, pentru faptul că ați fost un profesor exemplar, dar și pentru multe care nu sunt spuse aici, colegii de la Facultatea de Biologie și Geologie, împreună cu colegii de la Complexul Muzeal Bistrița-Năsăud vă mulțumesc și vă doresc multă sănătate și bucurii!

**La Mulți Ani, dragul nostru profesor!**

# BIOLOGY

# THE CHARACTERIZATION OF *STAPHYLOCOCCUS* SPECIES ASSOCIATED WITH HUMAN INFECTIONS

Diana Rodica MIREA*, Minodora Elena ROSALIM**, Rahela CARPA*

**Abstract.** *Staphylococcus* is the most frequent genus, from *Micrococcaceae* family, involved in human pathology. The appearance and evolution of *Staphylococcus* infections, as in any other infectious process, is conditioned by the aggressivity of the microbes, the defence properties or the receptivity of the attacked organism. Age matters too, children and teenagers beeing the most affected. The clinic aspect of the infection also depends on the way germs enter the body, their ability to spread, their location and the infectant dose.

**Key words:** *Staphylococcus*, infection, attacked organism, germs.

The name *Staphylococcus* was given by the Scottish surgeon Alexander Ogston in 1882 and it comes from the word "*staphylos*" which means bunch of grapes, due to incomplete separation of daughter cells from mother cells on solid cultures. On liquid cultures, the coci apear isolated a few or in small groups with irregular disposition (Humphreys, 2012; Ieremia et al., 1985).

This genus is formed of Gram-positive, catalase-positive, aerobe facultative anaerobe sphere-shaped coci, with sizes between 0,8-1 micron, which are divided in succesive perpendicular plans (Dorobăţ, 2006; Ieremia et al., 1985; Popovici et al., 1965).

The incidence of nosocomial infection with staphylococci increased in the last decades, because of the many injuries and the use of a high number of medical devices such as catheteres, those being development-friendly environments for this type of infection (Shinefield and Ruff, 2009).

The most frequent *Staphylococcus* infections are skin infections, but they often affect the respiratory or haematogenetic pathway, especially at elderly and children, even newborns (Ieremia et al., 1985).

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## 1. HABITAT AND TAXONOMY OF *STAPHYLOCOCCUS*

In 1974 there were 3 known species according to the International Taxonomy Committee: *Staphylococcus aureus*, *S. epidermidis* and *S. saprophyticus*. Then, in 1983 Kloos and Schleiber proposed a classification scheme of *Staphylococcus* which included 9 species, but that one was never officially established.

Coagulase-positive staphylococci were included in *S. aureus* species, usually haemolyticus, which caused the most infections in people. Human isolated, *S. aureus* strains form an homogeneous group.

*S. epidermidis* defined a heterogenous group of staphylococci that had a common property which was the absence of coagulase synthesis ability. The need of differentation in species resulted in diverse attempts to establish some new species or subspecies.

*S. saprophyticus* included coagulase-negative strains, *novobiocin-resistant*, that could have grown relatively slow in anaerobiosis, forming small glucose quantities. Due to this property, this species was included initially in *Micrococcus* genus and then reincluded in *Staphylococcus* genus because of the guanine and cytosine display in DNA (Ieremia et al., 1985). Nowadays, there are more than 40 species known of *Staphylococcus* (*Table 1*).

**Table 1.** *Staphylococcus* species (according to NCBI taxonomy)

| | | |
|---|---|---|
| *S. agnetis* | *S. fleurettii* | *S. nepalensis* |
| *S. arlettae* | *S. gallinarum* | *S. pasteuri* |
| *S. aureus* | *S. haemolyticus* | *S. patrasii* |
| *S. auricularis* | *S. hominis* | *S. pettenkoferi* |
| *S. capitis* | *S. hyicus* | *S. piscifermentans* |
| *S. caprae* | *S. intermedius* | *S. pseudintermedius* |
| *S. carnosus* | *S. jettensis* | *S. pseudolugdunensis* |
| *S. chromogenes* | *S. kloosi* | *S. rostri* |
| *S. cohnii* | *S. leei* | *S. saccharolyticus* |
| *S. condimenti* | *S. lentus* | *S. saprophyticus* |
| *S. delphini* | *S. lugdunensis* | *S. schleiferi* |
| *S. devriesei* | *S. lutrae* | *S. sciuri* |
| *S. epidermidis* | *S. lyticans* | *S. simiae* |
| *S. equorum* | *S. massiliensis* | *S. simulans* |
| *S. faecalis* | *S. microti* | |
| *S. felis* | *S. muscae* | |

*Staphylococcus* infection sensitivity appeares in all the human body tissues, the frequency beeing although determined by the pathway the coci choose to enter the body. The most frequent staphylococci infection is the cutaneous infection, because the skin pathogens have access to the hair follicle.

Also, another entering pathway is the sweat glands, especially those from axillary area. *Staphylococcus* infections can also be respiratory, mostly at newborns and elderly as a result of a flu or pneumonia. The infections that choose the haematogenetic pathway appear at children, grown-ups who suffered burns or different injuries and elderly treated with corticoids or subjected to intensive care. At children aged between 0-12 years old, the second most affected pathway after the haematogenetic one is the diaphysis of the growing long bones. Also, the enterotoxines can pervade in the digestive tube through a contamined product leading to a staphylococcal food poisoning (Ieremia, 1985).

It was proven that all the factors that reduce infectious resistance, allergic diseases, nutrition disorders and debilitating diseases, encourage the growth of staphylococci infections. Also, children and teenagers are more sensitive (Popovici et al., 1965).

There are 2 forms of epidemical *Staphylococcus* infections: nosocomial infection and food poisoning. The first one is conditioned by the presence of carriers, the circulation of multi-resistant strains beeing favored by the hospital environment. At temperatures between 20-37°C staphylococci synthesize enterotoxins and multiply in any food that gives them the necessary nutritious substrate. Commonly meat and dairy products are the most affected (Ieremia et al., 1985).

## 2. MORFOLOGY AND CELLULAR STRUCTURE

At staphylococci, the cell wall has a 3-layer structure and is formed of teichoic acids, peptidoglycan (murein) and an external layer of proteins. The thickness of the cellular wall is 20-80 nm, reversed with the age of the cell. After a few studies it was proven that more than 70% of the cellular wall is made of peptidoglycan and the teichoic acids are connected covalently to N-acetil-muramic residues of peptidoglycanes through a phosphodiester bond (Umeda et al., 1987).

Teichoic acids from *S. aureus* are composed of ribitol units and those from coagulase-negative staphylococci are formed of glycerol. Electronegative charged teichoic acids play an important part in mentaining the ionic environment which is needed for the synthesis and reproduction role of cation-dependent membrane through magnesium ions fixation. This type of acids are the essential chimical component of cell wall receptors for staphylococic bacteriophages.

Peptidoglycan has a reticular structure formed of alternative units of β-1,4-N-acetyl-glucosamine and N-Acetylmuramic acid, connected through tetrapeptidic units, which are tied up through pentapeptidic bridges formed exclusively of glycin. The main cause of intense fixation of basic dyestuff is the

chemical properties of peptidoglycan those being the foundation of tinctorial afinity at cells with intact wall (Codiță, 1993).

*Staphylococcus aureus* produces multiple molecules that can play a part in immune evasion, among these also being protein A. Immune evasion is a strategy which tumors or pathogene organisms use to avoid the hosts immune reponse, this way raising the probability of passing the pathogene agent to other hosts (Kobayashi and DeLeo, 2013).

Protein A is a surface proteic structure, very important in microbiological diagnosis. In human bacteriology it is considered a characteristic property of *S. aureus*. This protein is tightly connected to peptidoglycan in the bacterian wall structure, owning the property of pseudoimmune combining and being freely secreted in the external environment. This property is due to steric configuration with Fc fragment of IgG and offers multiple possibilities of using it in haematological, bacteriological and immunological studies (Codiță, 1993; Kobayashi and DeLeo, 2013).

The external proteic layer is formed of a succession of receptors that are responsible of bacterian cells properties such as: clumping-factor, which is a receptor for fibrinogen and receptors for fibronectin and collagen involved in species adhesion to organic substrate (Codiță, 1993).

The cellular membrane has a three-layer structure and is made of glycolipids, phospholipids and cardiolipin. It represents the residence of the transporting system made of unsaturated menaquinone and a and b cytochromes. There are also other enzimes like dehydrogenase and acid phosphatase, with cellular membrane activity (Codiță, 1993).

Staphylococcus nucleus is prokariotic, without membrane, made out of a unique DNA bicatenar molecule (Codiță, 1993).

Among the intracellular elements of staphylococci there are also mesosomes, cytoplasmic membrane structures, which contain reducing enzimes and enzimes from the respiratory chain. These play a part in cellular division (Codiță, 1993).

## 3. *STAPHYLOCOCCUS* SENSITIVITY STRAIN PATTERNS

Oxacilin and methicillin resistant strains, named MRS are actually resistant to all β-lactams, including cephalosporins and carbapenems. These strains, associated with hospital-acquired infections, are frequently multiple-resistant to other antimicrobial agents such as erythromycin, clindamycin (MLSBi) and tetracycline, while MRSA, associated with community infections, is frequently resistant only to β-lactams and erythromycin.

### a. Methicillin (oxacillin) resistance/sensitivity – MRS/MSS

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major concern for public health due to its abillity to cause severe human infections in both clinic environment and in community (Marigadas et al., 2017; Kong et al., 2016; Chiew et al., 2018). Infections with MRSA lead to a significant, additional weight for the medical system worldwide, with higher mortality and financial costs compared to MSSA (Kong et al., 2016; Chiew et al., 2018).

MRSA infections are an alarming factor, not necessarily because of the methicillin resistance, but because this *Staphylococcus* has resistance to many other chemotherapeutic agents. Nowadays, MRSA is a majorly pathogen agent in hospitals because of the patients who are hospitalized a long period of time, intravenous drug abuse and medical personnel who can be carriers of this *Staphylococcus* (Vidhani et al., 2001).

There are a lot of factors that encourage long exposure to MRSA such as: HIV infection among external hemodialysis patients, moving from one medical institution to another, previous exposure to fluoroquinolones, skin injuries, etc. (Chiew et al., 2018).

Acceptance of MRSA infected patients in hospitals is a major challenge regarding the safety of the other patients, because MRSA is easily transmitted in a medical environment. Many hospitals adopted policies regarding universal screening, this being embraced by the Tan Tock Seng Hospital from Singapore in 2012, as part of a program with the purpose of improvement the quality and control of hospital-acquired infections. In spite of that, this control method is usually made with a high operational cost and it is a burden for the capacity of workforce and hospital spots designed for patients (Chiew et al., 2018).

Other coagulase-positive and coagulase-negative methicillin-resistant staphylococci such as: *S. hominis, S. xylosus, S. epidermidis* and *S. haemolyticus* are responsible for a variety of oportunistic infections in human population, especially in immunity compromised patients (Becker et al., 2014; Buzón-Durán et al., 2017).

### b. Inducible clindamycin resistance – MLSBi

*Staphylococcus aureus*, coagulase-negative staphylococci and β-haemolytic streptococci can be naturally or induced resistant to clindamycin – MLSBi = macrolides, lincosamides, streptogramins B inducible phenotype. Using the difuzimetric method or the broth microdilution method, the inducible clindamycin resistance can be detected at *Staphylococcus.* The difuzimetric method uses clindamycin and erythromycin disks arranged one next to another. The *Staphylococcus* routine test is made by placing a 2 μg clindamycin microcomprimate at a distance of 15-25 mm from a 15 μg erythromycin

microcomprimate. The flattening of the *inhibition zone* around the clindamycin disk proximal to the erythromycin disk (D test) indicates a inducible clindamycin resistance. If however at some organisms appeares a small increase on the entire clindamycin inhibition zone, those organisms also need to be recognized as clindamycin-resistant, careless of the presence of D phenomenon (Lall and Sahni, 2014; Patel et al., 2006).

Clindamycin can be used for methicillin-resistant *Staphylococcus* treatments, but is also necessary in effectuation of the D test to exclude the clindamycin resistance (Navidinia, 2015).

## 4. *STAPHYLOCOCCUS* INFECTION IDENTIFICATION

According to some statistics from 1953, 70% of the human population were healthy carriers of *S. aureus* at some point in their lives (Leonida et al., 1973).

**Microscopic characteristics.** In the growing phase, *Staphylococcus* looks like a cell made out of a nucleus without membrane, mesosomes, polyribozomes and a three-layered cytoplasmic membrane. The cellular wall at young cells has a dense thickness that varies between 18-25 nm and is made out of peptidoglycan, teichoic acids and a proteic external layer. Due to the crossing of the peptidic part, the cellular wall has a dense structure in order to keep the globular form, resistant to bad environment circumstances (Ieremia et al., 1985).

**Biochimical characteristics.** The chemical composition of the nucleus is a unique, double-stranded DNA molecule (Ieremia et al., 1985).

Protein A (Cowan protein) is a major proteic component of the cellular wall at *S. aureus,* but it can be found in 90% of the pathogenic *Staphylococcus* strains, in different quantities. Protein A is missing from *S. epidermidis*. Studies have shown that this protein is able to react pseudoimmunely with the Fc part of certain classes of mammals immunoglobulins, among which are also the humans (Ieremia et al., 1985; Săcărea, 2006).

**Culture characteristics.** *Staphylococcus* can grow between 10-45°C with best development at 37°C, showing wide limits of termic tolerance. The ideal pH for pathogenic staphylococci is between 7-7.5, the same as the internal pH of human body. Despite this fact, the tolerance at pH values is high, between 4-9.3 (Ieremia et al., 1985; Popovici et al., 1965).

Nutrition necessities of *Staphylococcus* are minimal, but the pathogenic strains need a series of aminoacids and growing factors like thiamine and nicotinic acid, in the aerobic environment and uracil and a carbon source for fermentation, in the anaerobic environment (Ieremia et al., 1985; Leonida et al., 1973; Gillaspy et al., 2009).

The growing and development of pathogenic staphylococci on cultures is made quickly, the generation time beeing after around 30 minutes. On solid cultures, after around 18-20 hours of cultivation in the incubator, the pathogenic isolated colonies of *Staphylococcus* are circular, with regular edges, colored in pale yellow, in the shape of the letter „S'' (*Figure 1*). On solid cultures with blood, pathogenic *Staphylococcus* causes a circular hemolytic area around the colony (Ieremia et al., 1985).

**Fig. 1.** *Staphylococcus* on culture medium (https://www.netdoctor.co.uk/healthy-living/a5562/methicillin-resistant-staphylococcus-aureus-mrsa-infection/)

## 5. *STAPHYLOCOCCUS* SPECIES ASSOCIATED WITH HUMAN INFECTIONS

There are over 40 *Staphylococcus* species known until today, a few of the most frequent being: *S. aureus, S. hominis, S. epidermidis* and *S. haemolyticus*.

### a. *Staphylococcus aureus*

Gram-positive *Staphylococcus aureus* is a medically important pathogen agent of a variety of infections, from superficial skin infections to complicated bacterial tissue infections. *S. aureus*, especially antibiotic resistant strains, is usually associated with a high risk of morbidity and mortality in nosocomial infections as a result of severe sepsis and septic shock (Kong et al., 2016).

*S. aureus* strains are able to attach to mucosa, epithelium and endothelium. This feature comes as result of the structural and biochemical characteristics such as the presence of: lipoteichoic acid that helps attaching to epitheliums, protein A which helps attaching to human corneocytes, cellular wall receptor with proteic structure for fibronectin and clumping factor. The high amount of hydrophobic proteins and the presence of polysaccharide capsular structures on the surface of the cellular wall develops at strains the abillity to

colonize mucosa and conjunctive tissue, allowing them to attach to biomaterials and plastic materials such as sounds and catheters (Codiță, 1993).

*Staphylococcus aureus* is naturally widespread in the whole human body, with asymptomatic colonazation of skin and oral (*Figure 2*), respiratory and gastrointestinal systems (Olowe et al., 2007). Despite this fact, if this type of *Staphylococcus* survives, it can lead to a blood infection, gastrointestinal infection, or any other organ system infection that might threaten the life of a human being (Al-Anazi, 2009; Heijer et al., 2013; Haque et al., 2011; Pai et al., 2010; Shen et al., 2013).

Being one of the most frequently isolated nutritive pathogenic agents, *Staphylococcus aureus* is considered to be a major cause of food poisoning worldwide (Rubab et al., 2018).

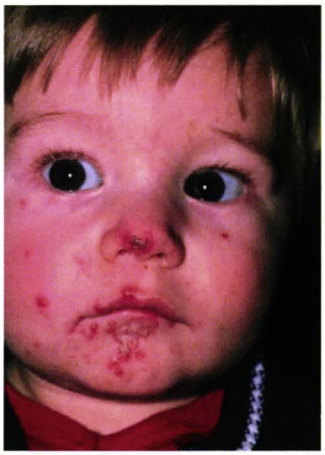

**Fig. 2.** *Staphylococcus aureus* infection
(https://www.merckmanuals.com/en-ca/home/infections/bacterial-infections-gram-positive-bacteria/staphylococcus-aureus-infections)

Nowadays, around 20-30% of human population are carriers of this type of staphylococci. They do not show any sign of infection and this way can spread this bacteria in the community. Some studies searched into a potentially involvement of this *Staphylococcus* as an ethiologic agent of autoimmune diseases (Mousavi et al., 2017).

**b. *Staphylococcus epidermidis***

*Staphylococcus epidermidis* is a Gram-positive bacteria and one of the most widespread causes of nosocomial infections due to its strong abillity to form microbial biofilms on catheters and surgical implants. The most common way of entering the body is through intravascular catheters (Hernandez-Montelongo et al., 2018; Vuong and Otto, 2002).

After completing numerous studies, it was found that the dezinfection of gloves worn by the medical personel can reduce substantially the risk of transmitting the bacteria when the gloves are worn for many stages of nursing at the same patient (Assadian et al., 2018).

*S. epidermidis* is a harmless commensal microorganism, that can be found on human skin (*Figure 3*), but which can become pathogenic when is found in blood or when it colonizes different medical devices. Oftenly, this infection is associated with infectious thrombosis (Ma et al., 2017).

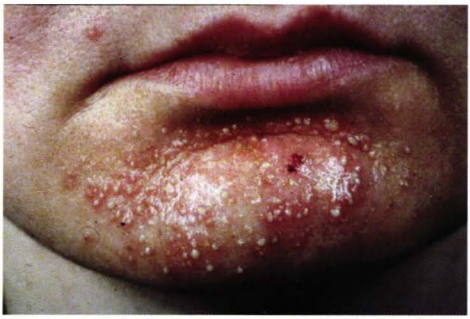

**Fig. 3.** *Staphylococcus epidermidis* infection
(http://www.dermweb.com/skininfectionsandinfestations/saureus3page.htm)

This type of staphylococci does not produce agressive determinants of virulence. Rather, the factors that usually support commensal lifestyle of *S. epidermidis* offer an additional benefit during infection, having a role in balancing the epithelial microflora and serving as a reservoir of resistant genes (Otto, 2009).

*Staphylococcus epidermidis* becomes very often the major pathogenic agent for patients with a compromised immune system like drug addicts, patients who follow immunosuppressive therapy, premature newborns and patients with AIDS (Vuong and Otto, 2002).

**c. *Staphylococcus haemolyticus***

*S. haemolyticus* is a Gram-positive bacteria, optionally anaerobic that develops on a wide range of substrates such as: glucose, maltose and sucrose (*Figure 4*) (Daniel et al., 2012).

From the coagulase-negative staphylococci, *Staphylococcus haemolyticus* is the secondly most isolated type from hemocultures, after *S. epidermidis*, having also the highest level of antimicrobial resistance (Barros et al., 2012).

Like its name says, this type of staphylococci is wide-spread in blood infections. In spite of all that, the molecular basis of hemolysis remains unknown (Da et al., 2017).

Another important characteristic of *S. haemolyticus* is its abillity to survive in hospitals. According to a study made in South Korea in 2011, MRSH

strains were present on 51.4% of x-ray cassetts in a radiology department (Czekaj et al., 2015).

**Fig. 4.** *Staphylococcus haemolyticus* on culture medium (https://www.flickr.com/photos/nathanreading/6797437217)

Numerous studies have shown that *S. haemolyticus* generates cytotoxins which kill the macrophages, a mechanism that is essential for bacterial colonization (Daniel et al., 2012).

**d. *Staphylococcus hominis***

*Staphylococcus hominis* is coagulase-negative bacteria being considered one of the three most commonly isolates found in the blood of the newborns and immunosuppressed patients. In the past years, the rates of bacteriemia induced by *S. hominis* infection, septicemia, endophthalmitis and endocarditis have increased in frequency. This type of staphylococci are often very resistant to antibiotics and as a result they are difficult to treat (Mendoza-Olazaran et al., 2015).

From the most considerable strains of coagulase-negative staphylococci, *Staphylococcus hominis* is ranked third in importance after *S. epidermidis* and *S. haemolyticus*. *S. hominis* is a varied species, from a genetic point of view and is believed that recombination plays a significant part in this diversity (Mendoza-Olazarán et al., 2015; Zhang et al., 2013; Szczuka et al., 2016a).

This bacteria can be responsible for blood infections, endocarditis, peritonitis, bone and joints infections (Kaufman and Fairchild, 2004; Szczuka et al., 2016b). *S. hominis* is a commensal bacteria of the human skin and at the same time an oportunistic pathogen (Piette and Verschraegen, 2009).

**e. *Staphylococcus xylosus***

*Staphylococcus xylosus* is ordinarily not a pathogenic species, often commensal on the skin of mammals and birds. This bacteria lives naturally in food, being one of the main cultures used for starting the meat fermentation process (Vela et al., 2012).

The cases where *S. xylosus* was discovered as being the primar pathogen agent in both human and veterinary medicine are rare. The infections lead to soft tissue abcesses and granulomas, with bacterial migration to internal organs (Gozalo et al., 2010).

**f. *Staphylococcus warneri***

*Staphylococcus warneri* was described for the first time in 1975 by Kloos and Schleifer. It can be a nosocomial pathogen, mostly being involved in catheter infections (Buttery et al., 1997; Kamath et al., 1992).

At immuno-compromised patients, *S. warneri* can lead to serious infections, including endocarditis affecting the heart valves. Also, among other conditions it can cause: vertebral osteomyelitis, urinary tract infections (UTI) and subdural empyema (Buttery et al., 1997; Kamath et al., 1992).

**g. *Staphylococcus saprophyticus***

Around 1970, after more than 10 years since the first identification of *S. saprophyticus* in urine, it became famous for causing the urinary tract infections. At young women, it is, after *E. coli*, the second most frequent pathogenic agent for UTI (Hovelius and Mardh, 1984).

Urinary tract infection caused by this type of *Staphylococcus* is almost all the time symptomatic, one of the risk factors for infection being the sexual contact because it facilitates the spreading of bacteria from the urethral and periurethral area into the bladder. As a result of a study, it was found out that almost 70% of the women with UTI caused by *S. saprophyticus* had sexual contact within 24 hours before the outburst of symptoms (Svanborg, 1998).

**h. *Staphylococcus auricularis***

*Staphylococcus auricularis* is a motionless, Gram-positive bacteria, with a diameter between 0.8-1.02 μm. This bacteria does not form germs and it can be found mostly in tetrades or pairs (*Figure 5*) (Kloos and Schleifer, 1983).

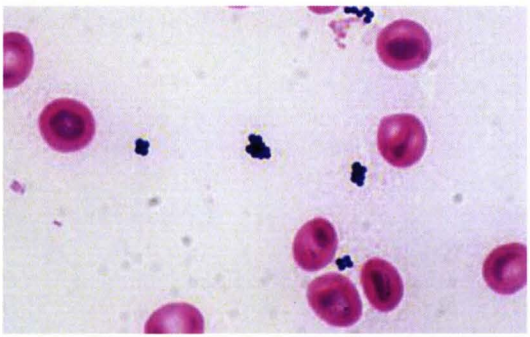

**Fig. 5.** *Staphylococcus auricularis* microscopic aspect (http://microbe-canvas.com/Bacteria.php?p=1144)

This type of *Staphylococcus*, like the most coagulase-negative ones, can cause many conditions, most frequently in the blood of patients with septicemia. It can also occur in endophthalmitis, respiratory diseases and at prematurely born babies (Sagar, 2011).

**i. *Staphylococcus capitis***

*Staphylococcus capitis* is a Gram-positive, coagulase-negative species, with potential of causing human infections and spreading in hospital environments, inducing 20% of the neonatal septicemia. This *Staphylococcus* can be found in a lot of infections acquired during surgeries or in the early postoperative period of time. Also, is spreads mostly in the limb and head area, especially in ears and forehead (Greco-Stewart et al., 2013; Tevell et al., 2016).

**j. *Staphylococcus simulans***

*Staphylococcus simulans* is another type of coagulase-negative staphylococci and also a well known pathogenic agent that affects mostly domestic animals like: cows, sheep, goats and horses. Isolated, in human infections, *S. simulans* is rare. Altough, if people come in direct contact with infected animals this represents a major health risk (Shields et al., 2016).

## CONCLUSIONS

*Staphylococcus* genus comprises over 40 species recognized by NCBI, which were identified based on cultural, microscopic, biochemical but mostly molecular characteristics. Out of the ones mentioned in this study, many are involved in the human nosocomial infections.

*S. capitis, S. auricularis, S. simulans* are rarely isolated from the clinical probes and they are granted a lower attention than *Staphylococcus* species which are more frequent like *S. epidermidis* and *S. haemolyticus*.

The 3 species of staphylococci (*S. aureus, S. epidermidis* and *S. haemolyticus*) are normally involved in: blood infections when catheters are used, osteomyelitis, bone and joint infections, endocarditis of the prosthetic valve, sepsis with early onset and skin infections caused by different injuries.

**Rezumat.** Genul *Staphylococcus* este cel mai frecvent gen, din familia Micrococcaceaelor, implicat în patologia umană. Apariția și evoluția infecțiilor produse de stafilococ, ca orice proces infecțios, este condiționată, pe lângă caracterele de agresivitate ale agentului microbian, de proprietățile de apărare sau de gradul de receptivitate al organismului atacat. Vârsta intervine de asemenea, copiii și adolescenții fiind mai susceptibili. Aspectul clinic al infecției mai depinde și de calea de pătrundere a germenilor în organism, de capacitatea lor de răspândire, de localizarea lor, dar și de doza infectantă.

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# THE EFFECT OF TWO LINGONBERRY EXTRACTS ON KIDNEY FUNCTION IN PARACETAMOL-INDUCED TOXICOSIS

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**Abstract.** The purpose of the study was to emphasize the protector potential of two kinds of *Vaccinium vitis-idaea* L (lingonberry) extracts respectively a 1:1 hydro-alcoholic extract (in a dose of 200 mg/100 g b.w.) and a dried extract 3:1 (in a dose of 100 mg/100 g b.w.) on some morphological parameters: the weight of the kidney; renal histology and biochemical blood parameters: creatinine and urea, in white Wistar rats intoxicated with paracetamol. Extracts were administered for 15 days in white female Wistar rats, weighing 135 ± 20 g alone, or at the same time with Paracetamol (75mg/100g bw).

The obtained results suggest that the two herbal extracts of *Vaccinium vitis idaea* in concentration of 200 mg and 100 mg d.s/100 g bw, respectively, in conditions of subacute paracetamol intoxication induce predominantly positive modulatory effects on the dynamics of the renal analyzed biochemical and morphological parameters, in particular for the 3:1 dried cranberry extract.

**Key words**: paracetamol intoxication, lingonberry extract, kidney morphology, rats.

## Introduction

Free radicals are molecules or atoms that have at least one uncoupled electron. These are unstable molecules that occur in the body as a result of various metabolic processes, immune reactions or the action of external factors, etc. The accumulation of excess free radicals generates, however, the oxidative stress that contributes to aging processes, and the mutations produced in the DNA molecule can influence carcinogenesis and many other diseases. In physiological conditions, ROS (reactive oxygen species) produced in the course of normal conditions are completely inactivated by cellular and extracellular defense mechanisms. This means that normally there is a balance between prooxidant (or oxidant) and antioxidant defense systems. In certain pathological conditions, increased generation of ROS and/or depletion of antioxidant defense systems leads to enhanced ROS activity and OS, resulting tissue damage.

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OS causes tissue damage by different mechanisms including promoting lipid peroxidation, DNA damage, and protein modification. These processes have been implicated in the pathogenesis of several systemic diseases including kidney. The kidney is an organ highly vulnerable to damage caused by ROS, likely due to the abundance of long chain polyunsaturated fatty acids in the composition of renal lipids. In recent years, OSs have become one of the most popular topics in research of molecular mechanisms of renal diseases. Antioxidant and reactive oxygen scavengers have been shown to be effective in animals for protecting the kidney, but it is hard to translocate these results to humans. (Ozbek, 2012).

*Antioxidants* are a group of compounds that can help support the integrity of cells against free radicals, unstable molecules that our body inevitably produces. Antioxidants are thus essential for the proper functioning of the body. They prevent cell and tissue damage where they act as traps. Following the presence of free radicals, the body puts into action all the defense mechanisms, those of prevention, physical and antioxidant defense (Jacob, 1995).

Antioxidants can be synthetic and natural. The main classes of natural antioxidants are: – pigments: polyphenols (flavonoids, phenolic acids, etc.), carotenoids, tannins, anthocyanins; – vitamins: vitamins that have important antioxidant properties are C, E and A; – enzymes and cofactors (glutathione, coenzyme Q10, etc.); – mineral salts (especially those of selenium, zinc and magnesium); – molecules that have the property of binding metals (albumin, transferrin, ferritin). The major benefits of antioxidants are: – they slow down degenerative processes and also strengthen the immune system. (https://www.secom.ro/articles/totul-despre-antioxidanti-ce-sunt-de-fapt-si-care-este-rolul-lor-major-in organism).

Recent research has shown that plant extracts and their phytoconstituents are effective free radical scavengers and inhibitors of lipid peroxidation (Dash et al., 2017; Yildirim et al., 2001). Over 50% of the drugs used today have their origin in natural products, a very wide range of basic drugs being isolated from approx. 90 plant species (Baker et al., 1995).

As concerning the *lingonberry* (*Vaccinium vitis-idaea* L.), it is known that its fruits have strong antioxidant activities. The suggested benefits of these fruits include the maintenance of vascular and view health, preventing or reducing the severity of cardiovascular diseases, and diabetes. cancer, and antimicrobial action. Lingonberry can prevent some diseases such as: urinary tract infections (Howell, 2002); renal calculi (kidney stones); diabetes; atherosclerosis; cancer, due to its content in carotenoids (β-carotene) (Hannum, 2004); respiratory viral infections – laboratory studies have shown a strong antiviral action of lingonberry leaves, which blocks the virus replication.

*Acetaminophen* (Paracetamol) is a widely used analgesic, antipyretic drug and is safe in therapeutic doses but accidental or intentional overdose causes liver and kidney disorders (Boutis and Shannon, 2001; Larson et al., 2005).

Paracetamol is primarily hepatotoxic and not least nephrotoxic. In vitro and in vitro studies have shown that the nephrotoxicity of analgesics is determined by an increase in ROS in the kidneys. Zhao et al (2011) showed that the consumption of analgesics increases ROS, nitric oxide and MDA levels as well as depletion of glutathione (GSH) in the rat's kidneys. The molecular mechanism for the induction of nephrotoxicity by APAP (acetaminophen) is poorly defined.

Although nephrotoxicity is less common than hepatotoxicity in acetaminophen overdose, urinary tract damage and disturbance of renal function may occur even in the absence of liver damages (Jones and Vale, 1993; Eguia and Materson, 1997).

Previous studies have shown that acute APAP overdose increases lipid peroxidation, endoplasmic reticulum stress, and suppresses antioxidant defense in renal tissue in experimental animals (Lorz et al., 2004; Ghosh and Sil, 2007; Abdel-Zaher et al., 2008). Because of kidney damages that APAP may produce, the antidote or treatment of renal toxicity is of toxicological importance.

Recovery of the kidney affected by various xenobiotics, including paracetamol, under the renal-protective influence of some natural medicinal products such as plant extracts, in our case the one obtained from *Vaccinium vitis idaea* L does not benefit from many data, being a reason for conducting this study.

That is why the purpose of our study was to evaluate the mode of action of 1:1 cranberry hydroalcoholic extract and 3:1 dry extract as well as their protective agents against paracetamol-induced kidney damage.

**Materials and Methods**

Experiments were performed on white female Wistar rats*, weighing 135 ± 20 g, divided into 6 groups of 7 animals each, as follows: control group (C); Paracetamol intoxicated group, 75 mg/100 g bw (P); lingonberry dry extract treated group (Ld) (100 mg extract/100 g bw); lingonberry hydroalcoholic extract treated group (Lha) (200 mg extract/100 g bw); P + Ld treated group (PLd) and P + Lha treated group (PLha). Plant extracts were administered by gavage, á jeun, for a period of 15 days.

The lingonberry fruit extracts, hydroalcoholic and dried) were obtained from fresh fruit harvested at Băişorii Mount (Cluj county) at the end of August 2014 at the UMF "Iuliu Haţieganu" Cluj-Napoca and were processed as described in a previous paper (Roman et al., 2017).

On the 16th day, animals were killed by decapitation after previous anesthesia with ketamine – xylazine cocktail (60:7.5 mg). After removing kidneys were weighed and the kidney was prepared for histological study with the usual methods (Mureşan et al., 1974) and blood samples were prepared for urea and creatinine determination.

Animals were obtained from the biobasis of „Iuliu Hatieganu" MPU, Cluj-Napoca and kept in a special space in compliance with the zoo hygienic and food conditions (standardized pellet for rodents, Cantacuzino Institute), water (*ad libitum*), light (12 h/day), heat, humidity, maintenance conditions complying according to the Law no.43/2014 on the protection of animals used for scientific purposes and to the 2010/63/UE Directive.

## Results and Discussions

### *Kidney weight*

After animals slaughtering, the kidneys were harvested and weighed, the results being presented in *Table 1.*

The weight of the kidney does not undergo statistically significant changes in group P, whereas in all other groups, the weight of the organ decreases by -12.69% (p = 0.015) in group Ld, by -15.74% (p = 0.01) in Lha group, by -22.94% (p = 0.002) in the PLd group and by -10.03% (p = 0.025) in the PLha group, compared to group C.

**Table 1.** Average kidney weight in the 6 experimental groups

| **Kidney weight (mg)** | | | | | | |
|---|---|---|---|---|---|---|
| | C | P | Ld | Lha | PLd | PLha |
| x±ES | 754,0±28,4 | 700,8±24,7 | 658±5,9 | 635,3±14,3 | 581,0±21,5 | 639,3±29,9 |
| n | 3 | 6 | 3 | 3 | 4 | 3 |
| p | - | 0,116 | 0,015 | 0,01 | 0,002 | 0,025 |
| D% | - | -7,05% | **-12,69%** | **-15,74%** | **-22,94%** | **-10,03** |

The table shows the average value ± standard error, n = no. of individuals/group, p – significant from 0.05 and the percentage difference from the control (D%).

The fact that the kidney weight decreased in the treated groups compared to the control we consider for group P, the administration of paracetamol for 9 days determined the kidney damage due to liver injury induced by oxidative stress generated by Paracetamol. In cranberry extract treated groups, because the administration has exceeded 10 days as indicated, the kidney undergoes structural and functional changes due to excessive urination, entering in a so-called state of functional fatigue, which is reversible after stopping the applied treatments.

### *Creatinine and serum urea*

The results of creatinine and serum urea are shown in Table 2. Following the applied treatments, there is an increase in the level of serum creatinine in all groups: significantly in group P (18.88%, p <0.006), Ld (25.55%, p <0.019) and by 27.77% (p <0.015) in group Lha compared to group C. Regarding the values of serum urea, they change significantly in group P, where it decreases by -34.62% (p <0.009) and increases by 39.20% (p <0.026) in group Ld and with 71.86% (p <0.0005) in the PLd group.

**Table 2.** Creatinine and urea levels in the six experimental groups

| | M | P | Ld | Lha | PLd | PLha |
|---|---|---|---|---|---|---|
| **Creatinine (mg/dL)** | | | | | | |
| x±ES | 0,9±0,04 | 1,07±0,04 | 1,13M0,009 | 1,15±0,09 | 0,97±0,06 | 0,93±0,06 |
| n | 6 | 6 | 6 | 6 | 6 | 6 |
| p | - | 0,006 | 0,019 | 0,015 | 0,186 | 0,325 |
| D% | - | **18,88** | **25,55** | **27,77** | 7,77 | 3,33 |
| **Urea (mg/dL)** | | | | | | |
| x±ES | 21,86±2,81 | 14,29±2,12 | 30,43±1,21 | 24,71±1,11 | 37,57±1,51 | 22,57±3,87 |
| n | 7 | 7 | 7 | 7 | 7 | 7 |
| p | - | 0,009 | 0,026 | 0,39 | 0,0005 | 0,396 |
| D% | - | **-34,62** | **39,20** | 13,03 | **71,86** | 3,24 |

The table shows the average value ± standard error, n = no. of individuals/group, p – significant from 0.05 and the percentage difference from the control (D%).

Dosage of serum urea as well as creatinine are tests used to assess kidney function or to monitor acute or chronic kidney disease. The creatinine and urea clearance calculated by assessing the concentration of creatinine and urea in the serum and blood is used to determine the rate of renal glomerular filtration, respectively, to test renal function. The concentration of creatinine and urea is an indicator of nephrotoxicity. The low clearance of creatinine and/or urea indicates a decrease in the ability to filter and elimination of excretion products from the blood into the urine in the kidneys. As creatinine clearance decreases, its blood levels increase, this being a diagnostic element in renal failure (Saka et al., 2012).

Regarding the assessment of serum urea level, it is well known that this is the excretion form of nitrogen contained in protein, and the amount excreted in the urine is directly proportional to the intensity of protein catabolism, as long as that renal function is unaltered. Serum values of urea are dependent on the degree of protein metabolism, diuresis – urinary excretion and renal function capacity. Low values of this parameter are found in children and pregnant women, in patients with severe liver disease who are hyperperfused, in cachexia or protein malnutrition.

***Kidney histology***

Following hematoxylin-eosin staining, histological study of the kidney in group C revealed the normal structure, characteristic for the glomerulus and renal tubular system. The renal glomerulus consists of the Bowman's capsule and the glomerular mesangium with podocytes. The subcapsular space does not show dilations or cellular elements, the unilayered glomerular epithelium being well highlighted. The tubular system, made up of the renal cortex level from the proximal and the distal tubules, has normal nephrocytes in terms of nuclear integrity and tinctorial properties.

The cells of the renal tubules also have a normal cytosolic appearance. The tubular lumen doesn't show dilation phenomena (*Fig. 1*). PAS staining revealed the

normal character of the analyzed renal structures. The positive PAS elements (basal membrane of the renal tubes, glycogen deposits, mucopolysaccharides, glomerular mesangium) have a normal distribution aspect (*Fig. 2*).

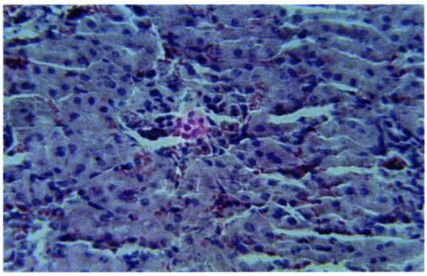

**Fig. 1.** Normal appearance of the kidney in group C (H&E, x400).

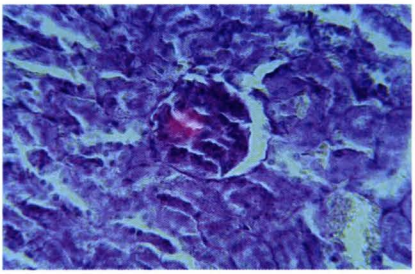

**Fig. 2.** Kidneys, group C, positive PAS elements are colored blue (PAS, x400).

In the paracetamol intoxicated group (group P) following the hematoxylin-eosin staining, the appearance at the level of the renal tubes of some territories of accentuated granular-vacuolar degeneration associated in some places with necroptotic foci is noticed. The renal glomerulus shows a slightly congested character. At the same time, it can remark phenomena of tubular epithelial desquamation. The nephrocytes nuclei have a slight hypochromic character (*Fig. 3*).

The staining of PAS-positive elements reveals that acetaminophen intoxication affects renal carbohydrate metabolism. Thus, a significant decrease in PAS-positive reserves can be noticed at the level of nephrocytes and then at the level of the glomerular mesangium. This is associated with thickening of the basement membranes of the proximal and distal tubule cells (*Fig. 4*).

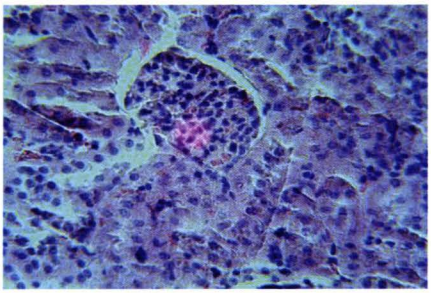

**Fig. 3.** Dystrophic and necroptotic appearance of paracetamol intoxicated kidney (group P) (H&E, x400).

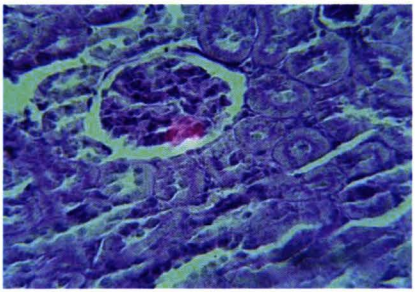

**Fig. 4.** Acetaminophenic poisoning (group P) decreases the abundance of PAS-positive elements in the kidney (PAS, x400).

The administration of lingonberry extracts (alcoholic extract, Lha group and dry extract, Ld group) does not negatively affect the structure of the renal cortex, so that both alcoholic and dry extract contribute to maintaining the morphological integrity of the kidney. There are normochromic nephrocyte nuclei, well-highlighted turgid nephrocytes with eosinophilic cytosol and the absence of epithelial desquamation. The subcapsular space is slightly dilated, but in this case, it has no histopathological relevance. (*Fig. 5, Fig. 6*).

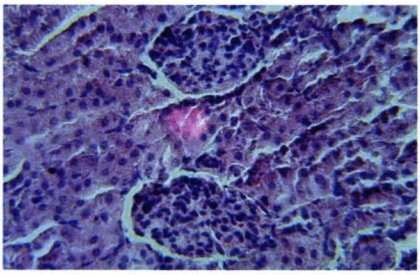

**Fig. 5.** Normal appearance of the kidney in the Lha group (H&E, x400).

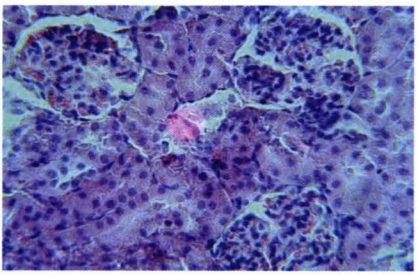

**Fig. 6.** The normal appearance of the kidney in the Ld group (H&E, x400).

PAS staining doesn't reveal noticeable changes in the basement membranes or in the abundance of PAS-positive elements. However, a tendency to decrease the abundance of PAS-positive elements is observed in the Ld group (*Fig. 7, Fig. 8*).

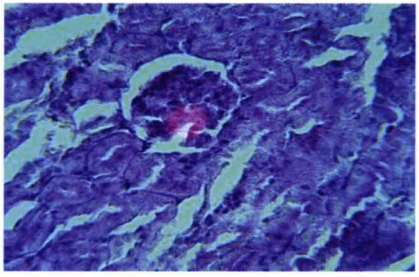

**Fig. 7.** PAS-normal appearance of the kidney in the Lha group (PAS, x400).

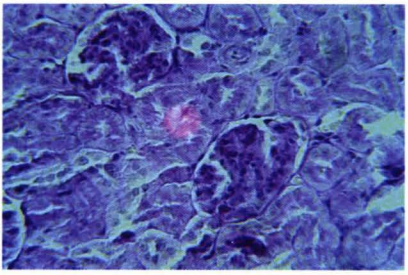

**Fig. 8.** The close to normal appearance of the kidney after PAS staining, Ld group (PAS, x400).

Concomitant administration of paracetamol and lingonberry extracts leads to a differentiated improvement in the histological (and also functional) appearance of the kidney. Thus, following the hematoxylin-eosin staining, in the PLha group, it is noticed that the extract contributes to maintaining a non-pathological aspect of the renal glomerulus and the tubular system. However, the PLha group also has rare tubular necroptosis, epithelial desquamation and mild glomerular congestion (*Fig. 9*).

Dried cranberry extract has a favorable histological action, against the background of chronic paracetamol intoxication. The protective/regenerative effect of the dry extract is more strongly highlighted at the morphostructural level than the effect of the alcoholic extract. In the PLd group, there is an aspect of the renal cortex close to that characteristic of a healthy kidney (see group C). The nephrocytes nuclei are normochromic, the epithelial desquamation is absent, the glomerulus is not congested and the tubular lumen has a normal appearance (*Fig. 10*).

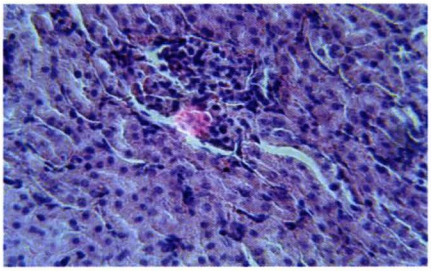

**Fig. 9.** The near-normal appearance of the kidney in the PLha group (H&E, x400).

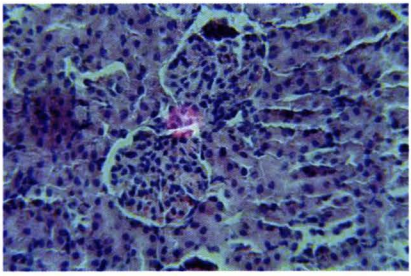

**Fig. 10.** The near-normal appearance of the glomeruli and renal tubules in the PLd group (H&E, x400).

In the PLha PAS staining group, a normal appearance and distribution of the positive PAS elements is highlighted both at the glomerular level and at the tubular level (*Fig. 11*). In contrast to the PLha group, the PLd group shows a marked depletion of mucopolysaccharides, glycogen and other PAS-positive substances in the tubular system and to a lesser in the renal glomerulus (*Fig. 12*).

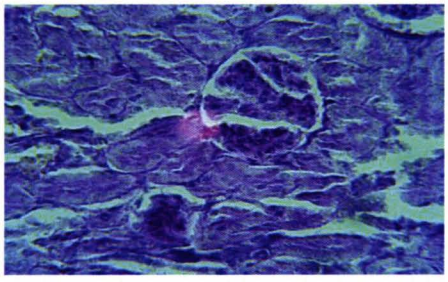

**Fig. 11.** Normal appearance of the kidney in the PMEx group (PAS, x400).

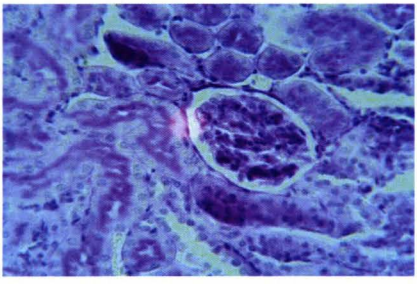

**Fig. 12.** Depletion of PAS-positive elements at the tubular level, PMp group (PAS, x400).

Following the analysis of the histological images of the kidneys, the harmful effect of acetaminophen on them can be ascertained, but without being of

a pathological nature. The association of the two extracts with the subchronic administration in a toxic dose of paracetamol reveals different protective effects, depending on the nature of the extract.

The toxicity of paracetamol on the kidneys is well documented (Loh and Ponampalan, 2006; Boutis and Shannon, 2001; Maze and Lee, 1998; Blakely and McDonald, 1995). According to the cited experimental data, paracetamol (N-acetyl-p-aminophenol) is metabolized primarily in hepatocytes and then, as the liver loses its detoxification capacity, paracetamol becomes metabolized in the renal parenchyma. The main metabolic system is the cytochrome system (cytochrome P-450 being predominantly involved) in the mitochondria. In the case of acetaminophen intoxication, mitochondria are severely affected and release oxygen and nitrogen radicals that intrinsically attack cellular structures against a background of major GSH depletion. Extrinsic attack cannot be ruled out by reactive species released by adjacent cells following membrane lipid peroxidation associated with increased phospholipidic bilayer permeability. Such events also occur in the renal tubular system, following hepatic insufficiency instauration, paracetamol having in addition to hepatotoxicity a relative nephrotoxicity, too (Cohen et al., 1997).

A secondary route by which paracetamol affects renal morpho function is the inhibition of COX-2 responsible for the synthesis of renal prostaglandins which in turn regulates the blood perfusion rate of renal parenchyma, renin release and excretion of Na + and H2O (hence the possibility of blood pressure problems in paracetamol intoxication).

The observed histological changes confirm the involvement of renal tubular cells in the metabolism of paracetamol, which leads to the hypothesis of centrilobular necrotic phenomena that induce the inability of liver detoxification, so that hepatic unmetabolized paracetamol reaches to the kidney where exerts its toxic effect. Thus, specific tubular necrosis/necroptosis occurs. At the same time, the decrease of glycogenic deposits and mucopolysaccharides in the kidneys is a consequence of acetaminophen intoxication, as mentioned by Hinson et al. (2010).

## Conclusions

The obtained results have shown that dry lingonberry extract has more obvious regenerative properties than the alcoholic lingonberry extract that if is administered in tandem with paracetamol in some aspects, may potentiate its destructive effect. Thus, the dry cranberry extract may be an alternative that has been shown to be effective in remedying paracetamol-induced renal disorders at a toxic dose. Therefore, we can consider that the dry cranberry extract could have much more obvious beneficial effects on the renal level in the case of ingestion of acetaminophen in therapeutic dose.

**Rezumat.** Scopul studiului a fost de a sublinia potențialul protector a două tipuri de extracte de *Vaccinium vitis-idaea* L (lingonberry), respectiv un extract hidroalcoolic 1:1 (în doză de 200 mg/100 g) și un extract uscat 3:1 (în doză de 100 mg/100 g) asupra unor parametri morfologici: greutatea rinichiului; histologici renali și parametri biochimici ai sângelui: creatinină și uree, la șobolanii albi Wistar intoxicați cu paracetamol. Extractele au fost administrate timp de 15 zile la femele albe de șobolan Wistar, cântărind 135 ± 20 g singure sau în același timp cu Paracetamol (75mg/100g bw).

Rezultatele obținute sugerează că cele două extracte din planta de *Vaccinium vitis idaea* în concentrație de 200 mg și 100 mg s.u./100 g respectiv, în condiții de intoxicație subacută de paracetamol induce efecte modulatorii predominant pozitive asupra dinamicii parametrilor biochimici și morfologici renali analizați, în special pentru extractul de merișoare uscate 3:1.

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# CONTRIBUTIONS TO KNOWLEDGE OF NESTING BLACK GROUSE (*TETRAO TETRIX*) IN RODNA MOUNTAINS NATIONAL PARK AND NATURA 2000 SITE (ROMANIA)

Claudiu IUŞAN *, Lukáš ZÁHOREC**

**Abstract**. The Rodna Mountains National Park and Natura 2000 site (ROSCI0125, ROSPA0085) is placed in the Eastern Carpathians, being a biodiversity sanctuary in the Carpathian Mountains. Among the rare fauna species found in this massif there is the black grouse (*Tetrao tetrix*), a sedentary bird species, breeding across northern Eurasia in moorland and bog areas near the woodland, mostly boreal, whose distribution is limited in Romania to the Maramureş, Suhard, Rodna, Călimani and Ţibleş Mountains.

The population estimation survey was conducted in the Rodna Mountains during the period 2004-2017, showing a population of 128 specimens distributed over an area of 47.000 ha. Twelve lek sites have been identified where the species performs its nuptial parade and a favorable conservation status has been assessed for 9 populations out of the 12 identified in the massive. A species monitoring protocol and a field data collection sheet used by the Rodna Mountains National Park Administration for the permanent assessment of the conservation status of the species have been developed. Among the identified threats are mentioned: over sheep folding and overgrazing, high numbers of shepherd dogs, change of land use, parasitic diseases.

**Key words**: black grouse, distribution, population.

## Introduction

Black grouse (*Tetrao tetrix*) is a galliform bird known for their complex courtship rituals. Males aggregate at traditional lek sites where they perform intricate vocal, visual or chemical displays to attract females and to defend their territories (Höglund&Alatalo, 1995, Sherman, 1999). They are birds of forest edge habitats and can be found at early stages of forest succession, moorlands and heaths (Storch, 2007, Lindstrom et al., 1998, Pulliainen, 1982). Black grouse use communal display areas called 'leks' to attract a mate, the blackcock (males) give a strange rhythmic call that is interspersed with a sneeze-like call to the greyhens (females), along with some cool dance moves (Niewold&Nijland, 1979, 1987, Klaus et al., 1990, Kasprzykowski, 2002). The best time to see this amazing site is during April and May.

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Black grouse is distributed across Eurasia, from Britain to Eastern Siberia. They occur as far Northas Norway (at 70°N) and as far Southas Kyrgyzstan and North Korea (at 40°N). This species has an extremely large range, and hence does not approach the thresholds for vulnerable under the range size criterion (Storch, 2007, Blotzheim, 1964). It declined significantly between 1970 and 1990, a trend that continued in 1990-2000 in most territories, except for Russia (Starling-Westerberger, 2001, Witherby et al., 1958). On the whole, it is considered that there is a trend of decreasing population. In Romania, the estimated population is 60-80 pairs. The largest flocks are present in Russia, Finland, Sweden and Norway (Glanzer, 1980, Loneux&Ruwet, 1997, Kamieniarz, 1997).

The black grouse is a species characteristic of areas at the upper limit of mountain ranges with rare trees. The male is smaller than the mountain cock (*Tetrao urogallus*) and in flight it is seen white stripes on the wings. Adults have different looks. The male has a glossy, blue-black plumage and twisted tail poles in the form of a lira, which in flight break out in the fork. The woman is brown. Feeds with leaves, buds (preferring birch buds), forest seeds, fruits, insects and their larvae (Faragó, 2002, Wegge&Kastdalen, 2008, Wubbenhorst&Pruter, 2007).

Before this study, there were only observations regarding the presence of black grouse in Rodna Mountains without the estimation of local population.

**Materials and methods**

The study area is Rodna Mountains National Park, which was designated also a Natura 2000 site and the black grouse is a key species for the site. It is Romania's second largest national park and, in administrative terms, it stretches across the Bistrița-Năsăud and Maramures counties. It is also one of Romania's three biosphere reserves, alongside the Retezat National Park and the Danube Delta. The Rodna Mountains National Park is located in the north of the Eastern Carpathians, incorporating just a part of the Rodna Mts. chain. It covers more than 47,000 hectares, with 80 percent of the area lying in the Bistrița-Năsăud County. The only settlement inside the national park is the Valea Vinului (Wine Valley) village of the Rodna village (Bistrița-Nasaud County), plus seven hectares of the built-up area of the Borsa town (Maramures County). A spectacular wild landscape greets in Rodna Mountains. Rich in waterfalls, glacial lakes, mineral water springs and caves, limestone areas; they form a vast imposing citadel full of charm and mystery.

The black grouse was mentioned as being present in Rodna Mountains by Filipaşcu (1974, 2004), the real status and distribution is under assessment by the Rodna Mountain National Park Administration since 2004. The thirteen field campaigns for assessment of distribution of the black grouse in Rodna Mountains National Park were carried out between 2004-2017 by the Park

Administration, being organized 10-15 field visits for identifying the reproduction habitats from sub-alpine and alpine area, during the breeding season of April-May.

The observations were realized by using an ornithological transects along 1 km. Two or three rangers walked during the transect and listen the individuals and recorded the observed and listen individuals. Some factors such as the very changeable weather with rains and snowing periods, foggy days, presence of wolves and foxes in the lek habitats, difficult access to alpine and isolated habitats (to 2.000 m altitude) represented a high challenge for the assessment process of black grouse (*Tetrao tetrix*) in Rodna Mountains.

For identifying the habitats of reproduction, working groups with the land owners, land administrators from Administrative Council of the National Park, forest engineers, foresters, hunters and volunteers were established. The field equipment used was represented by the binoculars, ornithological scope, photo lens (Nikon 600 mm), digital camera (Nikon D3X), camouflage wildlife tents, GPS units (Trimble Nomad), maps, aerial images thermal cameras, infrared monocular. Count methodology was similar across areas, being undertaken in accordance with the standardized methodology for lek counting (Baines, 1996; Hancock *et al.*, 1999; Sim *et al.*, 2008). Most of the observations were taken during the April-May in the lek sites, especially between 6-9 hours in the morning. Some investigated habitats were surrounding sheepfolds, high up in the mountains and there were seen individuals inside of anthropic habitats.

**Results and discussion**

In Rodna Mountains National Park, most of the lek sites were identified in the subalpine and alpine area, especially in the mixt habitats of dwarf mountain pine (*Pinus mugo*), common juniper (*Juniperus communis*), Swiss pine (*Pinus cembra*) and open pastures.

The broad habitat types used in the study and the Land Cover habitat classes which they are comprised: heather moorland (heather – vegetation that has >25% heath. Generally it occurs on welldrained, nutrient poor, acid soils.

Heather grassland -vegetation that has >25% heath but spectral differences allow separate classification from bog- vegetation dominated by cotton grass (*Eriophorum*) which occurs on deep peat), acid grassland (acid grassland -vegetation dominated by grasses and herbs on a range of lime-deficient soils). Rough grassland -mix of areas of managed, low productivity grassland, plus some areas of semi-natural grassland, which could not be assigned confidently to neutral, calcareous or acid grassland): Broad-leaved woodland (broad-leaved woodland -characterized by vegetation dominated by trees >5m high when mature, with tree cover >20%. Scrub (<5m) requires cover >30 % for inclusion. It includes stands of both native and non-native broad-

leaved trees and yew), Conifer woodland (coniferous woodland – characterized by trees >5m high when mature, forming a canopy >20%. Includes semi-natural stands and plantations), freshwater-includes standing open water such as lakes, meres. Also includes rivers and streams from bank top to bank top or to the extent of the mean annual flood, mountain habitats, range of vegetation types in the mountain zone characterized by prostrate dwarf shrub heath, sedge, rush and moss heaths.

The lek habitats for black grouse (*Tetrao tetrix*) and number of individuals recorded during 2004-2017 is presented in the *Figure 1.*

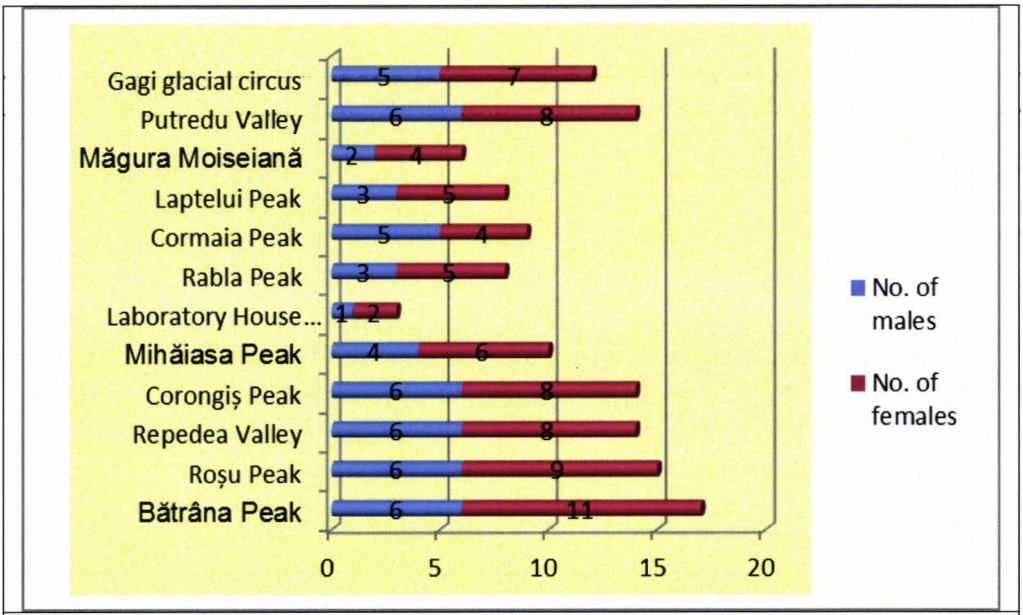


**Fig. 1.** Number of individuals identified in each lek from Rodna Mountains National Park (2004-2017)

The lek with high number of individuals are placed in habitats with a very difficult human access, such as Bătrâna Peak, Roșu Peak, Putredu Valley, Corongiș Peak and in some area with a high anthropic impact like Măgura Moiseiană, a low number of individuals were recorded. In some cases, such as Laboratory House from Pietrosu Mare, there was recorded a high number of dead individuals, possible from disease.

The distribution map of lek habitats from Rodna Mountains National Park is presented in the *Figure 2.*

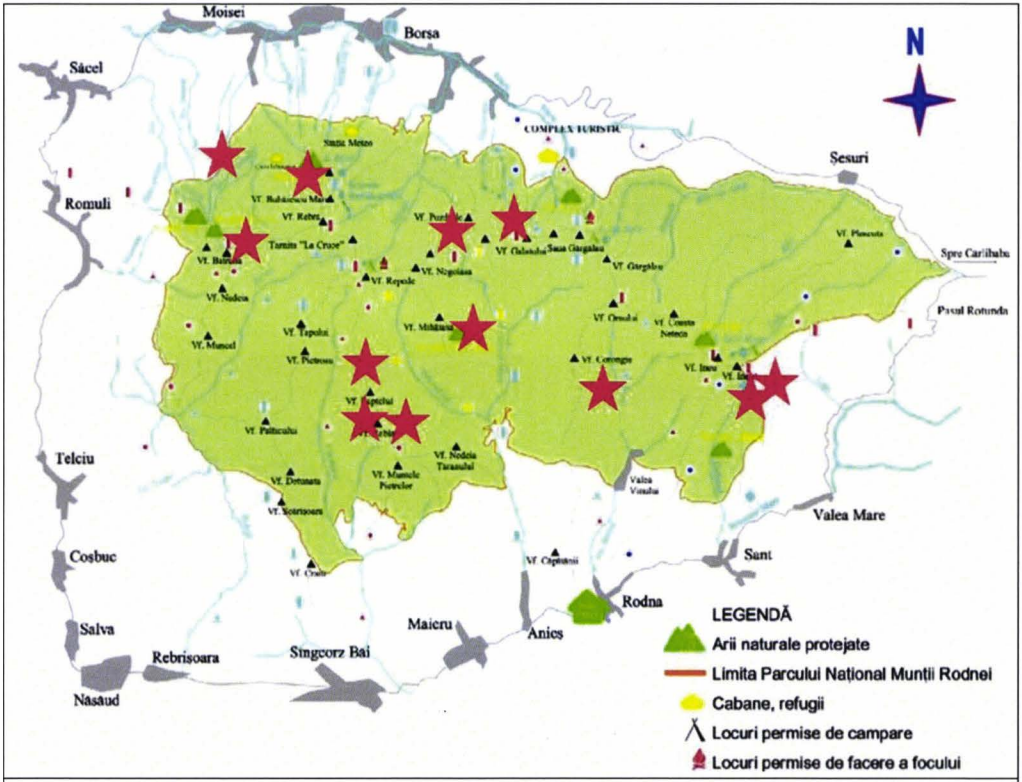


**Fig. 2.** Distribution map of lek habitats for black grouse (*Tetrao tetrix*) from Rodna Mountains National Park

According to the map of distribution, the lek is present mainly along the main ridge of Rodna Mountains, especially between 1700-2000 m altitudes.

Regarding the negative impact on black grouse population, there were registered some factors (*fig. 3*). The level of impact was assessed between 0 to 2 points, minimum being 0 and maximum being 2.

The overgrazing with sheep and high number of shepherd dogs are the main factors who are threatening the local population of black grouse. Taking into account that in Rodna Mountains National Park are 110 sheepfolds and everyone is having an average of 6 dogs, there are approximates 660 shepherd dogs which are running to catch the black grouse and in some cases are killing them. Having in mind that in last 10 years, the number of sheep has increased because of subsidies received from European Union as a payment for good practices of grazing in mountain areas, a high impact is recorded in some lek habitats.

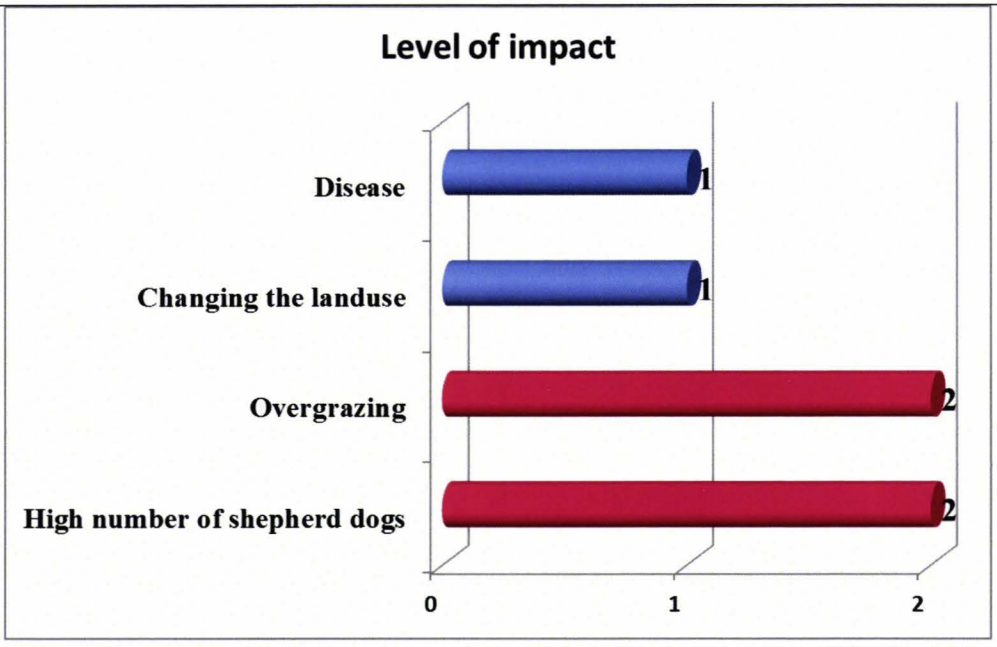


**Fig. 3.** Level of impact on black grouse in Rodna Mountains National Park

## Conclusions

During the twentieth century, black grouse have become species of major conservation concern throughout Central and Eastern Europe. National and European Union level policy (e.g., Natura 2000 protection) is directed at stabilization and recovery of the remaining populations. From this reason, the black grouse (*Tetrao tetrix*) is a key species for Rodna Mountains Natura 2000 site.

Most conservation efforts, however, have failed to reverse negative population trends, and the question of whether the causes of decline have been sufficiently understood remains (Doenecke et al., 1970, Storch, 2000 a,b). Regional differences in timing and extent of the decline may allow the exploration of alternative research hypotheses, but unfortunately, historic data are scattered and not readily available for analyses across landscape and regional extents. The spatial and temporal patterns that become apparent from historic population reconstructions may allow important insights into overall and site-specific causes of decline and extinction.

Knowing habitat use is important for precise wildlife management at appropriate scales, and that management should be knowledge-based, therefore we need both reliable data as well as knowledge about species. We have evaluated data that serve as a basis for management of black grouse in Rodna Mountains in order to obtain a better understanding of how black grouse are related to habitat. This is important because habitat alteration is one of the main

threats to species diversity and maintenance (Kålås et al., 2010, Beeston et al., 2005).

In the period 2004-2017 we conducted a survey of black grouse (*Tetrao tetrix*) in Rodna Mountains National Park and Natura 2000 site and were recorded 128 individuals in 12 lek sites, covering a surface of 47.000 ha in Eastern Carpathians. This is the first primary assessment study of black grouse (*Tetrao tetrix*) in Rodna Mountains National Park and we consider as being a baseline for future monitoring and evaluation of conservation status. The very changeable weather conditions and difficult access in lek habitats from alpine areas are discourage factors for managing an effective monitoring plan for black grouse in Rodna Mountains National Park.

Woodland or scrub (*Pinus mugo*) is often important to black grouse, providing sources of food and cover, and where conditions are suitable (e.g. open tree canopy and low grazing pressure) providing suitable field-layer vegetation. Black grouse distribution in Rodna Mountains appears to be closely associated to the presence of trees or woodland.

We tried to detect landscape characteristics that influence and generate differences in density among areas. This can also be used to predict impact of habitat degradation on the black grouse populations, because it is possible to relate a specific habitat component to demographic rates. Since habitat diversity was important it appears grouse are able to cope with degradation in their landscapes, as long as diversity is maintained at the appropriate scale. There were identified the factors which are threating the black grouse population in Rodna Mountains such as high number of shepherd dogs and overgrazing. The management measures of the Park Administration will be focused on controlling these factors.

Future research of the population of black grouse in Eastern Carpathians should focus on more extensive sampling of this area, including its western, southern and Eastern boarders, where other populations are (Maramures Mountains, Suhard Mountains, Tibles Mountains, Calimani Mountains).

**Acknowledgements**

Thanks to all volunteers who have provided all the data on black grouse, to all rangers of Rodna Mountains National Park for survey.

**Rezumat.** Parcul Național și siturile Natura 2000 Munții Rodnei (ROSCI0125, ROSPA0085) sunt situate în Carpații Orientali, fiind un sanctuar de biodiversitate la nivelul lanțului carpatic. Printre speciile de faună din masiv, se numără și cocoșul de mesteacăn (*Tetrao tetrix*), o specie de pasăre sedentară, răspândită în zona nordică a Eurasiei, în habitate de mlaștini, turbării, în apropiere de pădurile boreale, a cărui distribuție este limitată în România la nivelul Munților Maramureșului, Rodnei, Suhardului, Călimaniului și Țibleșului.

Studiul de estimare populațională a efectivelor cocoșului de mesteacăn (*Tetrao tetrix*) s-a derulat în Munții Rodnei de-a lungul anilor 2004-2017, observându-se 128 exemplare distribuite pe o suprafață de 47.000 ha. S-au identificat 12 locuri de rotit unde specia își desfășoară parada nupțială anuală. S-a evaluat o stare de conservare favorabilă pentru 9 populații de cocoș de mesteacăn din cele 12 populații identificate. De asemenea, s-a elaborat un protocol de monitorizare a speciei și un formular de colectare a datelor din teren de către personalul Administrației Parcului Național Munții Rodnei pentru evaluarea permanentă a stării de conservare a speciei. Printre amenințările identificate la adresa speciei în aria protejată, se pot menționa: supratârlirea, suprapășunatul, numărul ridicat al câinilor ciobănești, schimbarea categoriei de folosință a terenului, boli parazitare.

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# DIFFERENCES BETWEEN THE TESTING OF THE TOXICITY FOR CHEMICALS AND NANOMATERIALS

Andreea CRINTEA*, Anelisa-Marina SELEJAN**

**Abstract.** Human beings can come in contact with nanoparticles as they are obtained by natural processes. The main reasons for the environmental release of nanoparticles are the production, use or disposal of products that contain specific nanoparticles. The human organs that are more susceptible to external factors are the lungs and gastrointestinal tract. Due to the nanoparticles size, comparable with that of viruses, they can easily reach the bloodstream. Nanoparticles deposition in the central nervous system could induce adverse effects of cardiac rhythm, respiration and body movements – Silver nanoparticles accumulated in blood can cause edema and necrosis.

**Key words:** toxicity, nanoparticles, nanomaterials, medical application.

## Introduction

Advances in research on nanoparticles (NPs) and their use in numerous biomedical applications, led to finding new solutions to the various problems that had no solution. The basis of many of the main technological innovations of the 21st century is nanotechnology. Nanotechnology provides a chance in regard to changing and developing properties may have special applications in diagnosis. Nanotoxicology can be considered a new branch of toxicology and refers to the adverse health effects caused by nanoparticles. Nanomaterials are materials of which a single unit is sized between 1 and 1000 nm but is usually 1- 100 nm. Nanomaterials are used increasingly more and is very important to know the effects they have on ecosystem and human health. Manufactured nanomaterials will enter into the environment through intentional and unintentional releases. Nanomaterials in paints, fabrics will enter the environment proportional to their use and emitted nanomaterials will deposit on land or water.

Regarding nanomaterials with very small size, inhalation exposure can occur to airborne particles composed of nanomaterials covering a size range from a few nanometers to several micrometers in diameter. Nanomaterials may agglomerate into larger particles and may change their properties and may modify their behavior in the indoor and outdoor environments as well as their potential exposure and entry into the human body.

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Finally, substances can be toxic for human health or environment at different expose levels 1 (Hougaard et al., 2015; Sambale et al., 2015; Li et al., 2012).

Nanoparticles (NPs) can have beneficial effects on human beings, but instead of those positive effects regarding human health, scientists published different articles that suggest that certain nanoparticles can have negative effects on humans due to their size and properties. NPs can be very mobile in human body but also in the environment due to their small size. For example, NPs can access our body by inhalation, oral ingestion or skin contact and after they enter in our body they can disseminate to different body tissues. NPs in the air can remain suspended for a long period. Humans can be exposed to airborne NPs in an uncontrollable manner; NPs can also be disposed to the environment in liquid solution (Kalantzi and Biskos, 2014; Bohme et al., 2017). Different commercially NPs were studied regarding their cytotoxicity. Studies suggested that the major effects regarding cytotoxicity were in keratinocyte cultures and fibroblast ones. There was also evaluated the toxicity for metallic NPs, silver NPs with different sizes using a rat liver derived cell line, toxicity of different NPs on a mouse cell line with spermatogonial stem cell characteristics etc. The main effects were: mitochondrial function affected, cell apoptosis. Silver NPs were detected in many organs but for example, in spleen, kidney, brain, heart small concentration of these NPs were detected compared to nasal cavities were high concentration were detected. When rats received an aqueous suspension of agglomerated ultrafine silver NPs, a certain quantity of these agglomerates remained insoluble in the alveolar macrophages and in the septum for 7 days. There was observed a rapid clearance for the agglomerates, a mechanism who can be responsible for this suggested that even if agglomerated silver NPs remain insoluble in alveolar macrophages, ultrafine silver NPs were rapidly dissolved in the lung so silver can enter the blood capillaries by diffusion. Other experiments that were made to test the toxicity and biocompatibility of silver NPs were using zebrafish embryos. Spherical silver NPs synthesized by reducing $AgClO_4$ with reducing agents were used to treat the embryos and the embryos were monitored up to 120 h post fertilization. The abnormalities of zebrafish were due to the dose of silver NPs (Durán et al., 2010).

Silver NPs induce a dose-dependent toxicity in zebrafish embryos. The range for silver NPs is from 10 to 200 nm but most silver NPs have 20 nm and approximate 1% of silver NPs are found as silver ions. 1% of total silver in silver NPs was present as silver ions. Silver NPs toxicity is mediated by silver ions and the interactions of these particles with algae influences the toxicity of silver NPs. The toxicity of NPs was also studied using normal human lung fibroblast cells and human glioblastoma cells. The results of this experiment suggested modifications in cell morphology, viability, metabolic activity and oxidative stress. A possible mechanism responsible for those effects is believed to be the

disruption of the mitochondrial respiratory chain by silver NPs. The disruption of the mitochondrial respiratory chain by silver NPs lead to DNA damage. The immunological response of macrophages was investigated *in vitro*. Part of negatively charged gold NPs might absorb serum protein and then enter the cells *via* complicated pathways. This fact means higher cytotoxicity and immunological response of gold NPs compared to silver NPs. Silver NPs may also produce neurotoxicity because of the generation of free radical-induced oxidative stress and also by altering gene expression – apoptosis in developing neurotoxicity (Ray et al., 2009)

NPs of different sizes and specific chemical composition can be synthesized by different methods such as wet-chemistry or aerosol-based methods. Through wet- chemistry methods ionic solutions are formed and under certain conditions the nucleation of ions is initiated. On the other hand, through aerosol-based methods bulk materials are evaporating, the vapors are cooling to form clusters and NPs by nucleation and condensational growth. NPs which are generated through these methods can be collected by filtration or other techniques or can be collected in the form of powders which are very used for toxicity tests (Bohme et al., 2017; Hofmann et al., 2002).

**Toxicity methods**

Cell cultures are used to assess the toxicity for many different compounds. There are *in vitro* methods who can be used for assessing the toxicity: methods to test the integrity of cell membrane (LDH activity), bioluminescent methods. The most used assays to assess NP cytotoxicity are: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a useful method for measuring cell growth in cytotoxicity studies, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-etrazolium) and LDH to determinate cellular cytotoxicity and cytolysis (Bohme et al., 2017).

The mechanisms regarding interaction between NPs and living systems are not fully understood. The complexity refers when NPs have the ability to bind and interact with biological matter. Cells can take up NPs via active or passive mechanisms. There are many variables to take into consideration when working with nanomaterials: material, size, shape, concentration etc. Toxicity can vary in severity and depends for example on the mode of administration. If NPs are localized in lysosomes, mitochondria etc., they can cross the membranes of organelles. Different NPs are able to cross the blood-brain barrier through intravenous administration. After the administration they are able to be accumulated in the brain and can cause neurotoxicity. For example, *in vitro* studies regarding Superparamagnetic iron oxide nanoparticles (SPION), have shown the capacity of this NPs to reduce reactive oxygen species (ROS) generation, impair mitochondrial function etc. Scientists have used fibroblasts

cultures to demonstrate the ability to tune particle toxicity according to particle coating (Li et al., 2012).

Using Electric Cell-substrate Impedance Sensing (ECIS) was investigated the toxic effect of silver NPs on 4 different mammalian cell lines by studying the mitochondrial activity. Cultivating the cells in the presence of particles means that cells suffer apoptosis while cells cultivated with ions die due to necrosis. For the lung, the uptake of particles takes place in the alveoli and depends on the deposition of the NPs.

Regarding placentation, this defers between species. To compare data on transplacental transfer of NPs to the fetus, is important to know and understand the anatomical and physiological differences in placentation between humans and animal models. *In vitro* models of the placenta can be used for transplacental transfer of NPs. One of these models is Transwell. In this model, the membrane separates two liquid compartments. On both sides of the membrane cells can be grown (epithelial and endothelial cells to mimic blood vessels). Human placental perfusion models mimic the maternal and fetal blood circulation in the placenta by perfusing a single cotyledon. Through microscopy analysis, the size and the concentration of NPs can be measured.

On the other hand, through TEM (Transmission Electron Microscopy), physiochemical properties of NPs can be found. Atomic Force Microscopy (AFM) is another technique used to measure NPs. The concentration of airborne NPs can be measured using optical or electrical methods. The main advantage using electrical technique is that particles of any size can be detected. Other method is Differential Mobility Analyzer (DMA) who can be used to determine the size of nanoscale particles (Hougaard et al., 2015; Bohme et al., 2017).

On the other hand, toxic compounds can be classified due to their chemical nature, mode of action or class. The exposure class classifies these compounds as occurring in food, water, air and soil compared to the use class which classifies drugs in drug of abuse, therapeutic drugs, agriculture chemicals, cosmetics, pesticides, phytotoxins, food additives. Every study that is made on animals or cell lines, tissues, for testing toxicological effects of different compounds must be approved by Institute Animal Ethics Committee (IAEC).

There are different grades of toxicity that can be tested. Testing the acute toxicity on animals is important to find out the effects of a single dose on a particular animal species. For this test two different animal species are needed and the product that is investigated is administrated at different dose levels. The effects of the product can be observed during the next 14 days also the different changes in the dead animals must be investigated. Through this test the lethal dose of different products can be determinate but for this type of study more animals are needed and death ratio is high (Stolzenberg-Solomon et al., 2009). Because of this limitation there are different methods that were developed such as FDP (fixed dose procedure), ATC (acute toxic category), UDP (up-and-down):

for example, the FDP method can determine the nonlethal dose, the UDP method is favored because of the small number of animals that are used in the study. For acute inhalation toxicity tests rats are the most used. This test is performed for aerosol-like preparations. Also in this test the death animals are studied for different changes such as histological ones etc.

For topical preparations the eye irritation test and skin irritation test are the most important.

For example the Draize eye irritancy test or the Draize skin irritancy test are used to measure the negative effects of pharmaceutical substances in rabbits or guinea pigs. Guinea pigs are also used in skin sensitization test.

For example in murine local lymph node assay substances are applied on the surface of the animal ears during three days. After three days is measured the proliferation of lymphocytes in the draining lymph node. Repeated dose toxicity testing is another type of test that is carried out for a minimum of 28 days.

This test has different steps such as:

- The test substance is administered daily – oral route or parenterally, regularly;
- A rodent of any gender, age 5-6 weeks is used for repeated dose toxicity testing;
- Individual variation between the animals – weight ± 20%;
- A satellite group may be included;
- Behavioral and biochemical parameters of the animals should be recorded;
- At the end of the study, tissues from most of the organs are removed, and histological changes are recorded;
- Immunotoxicity studies can be performed (Kimm-Brinson and Ramsdell, 2001; Parasuraman, 2011; Test Method Evaluations, 2017, Stolzenberg-Solomon et al., 2009; Pankaj et al., 2013).

Mutagenicity testing is useful to determine changes in the sequence of DNA, chromosomal aberrations or other aberrations in DNA structure. Transgenic animals are more appropriate to evaluate the toxicity of different substances. Another test that are used for toxicity testing are sub chronic oral toxicity testing, chronic oral toxicity testing or carcinogenicity tests (Parasuraman, 2011).

No matter what study is, at the end is very important to notice the pathological changes and histopathological studies. At the end of one-generation reproduction toxicity test the number of dead and live pups has to be noted. On the other hand, live pups are weighed in the morning and in the evening every single day during the first four days. In the two-generation reproduction toxicity studies, necropsies, histological examinations must be carried out and at the end of the study, all animals should be sacrificed and different types of examination be made. The neurotoxicity studies are made using rodents and are important for

the evaluation of histopathological and behavioral neurotoxicity of chemicals. Are also important for the characterization of neurotoxic responses. Embryotoxicity studies can be made by using *in vivo* and *in vitro* methods. Rodents are mainly used for *in vivo* toxicity tests. This test can be performed also by using *in vitro* methods such as the embryonic stem cell test for embryotoxicity etc. Also in genetic toxicity test rodents are preferred and through this test can be identified gene mutation or chromosomes alterations (Kimm-Brinson and Ramsdell, 2001; Parasuraman, 2011; Test Method Evaluations, 2017, Stolzenberg-Solomon et al., 2009).

## Conclusions

Meaningful results on the toxicity of nanomaterials are only achieved when the conditions of exposure are realistic and is very important to understand how different methods of exposure of the nanoparticle might produce different results.

It is very useful to understand the assay and immunological evaluation can be an example of an area where nanoparticles interfere with conventional assays – the more complex structures and physicochemical properties typical of nanoparticles increases the probability of interference with reagents and outcome of assays.

Several studies conclude that response to NPs is diverse – extent of toxicity is to be measured in term of mortality rate, hatching, heart rate and abnormal phenotypes, nuclear deposition is believed to create a cascade of toxic events leading to DNA damage and similar ones, toxicity end points reveal a concentration-dependent occurrence of negative events such as death.

**Rezumat.** Oamenii pot intra în contact cu nanoparticulele ce sunt obținute prin procese de sinteză naturale. Principalele motive pentru eliberarea nanoparticulelor în mediu este producția, utilizarea sau înlăturarea produselor care conțin nanoparticule specifice. Organele umane care sunt mai susceptibile la factorii externi sunt plămânii și tractul gastro-intestinal. Datorită dimensiunii nanoparticulelor, comparabile cu cea a virusurilor, ele pot ajunge cu ușurință în fluxul sanguin. Depunerea nanoparticulelor în sistemul nervos central ar putea induce efecte adverse ale ritmului cardiac, respirației și mișcărilor corpului – nanoparticulele de argint acumulate în sânge pot provoca edem și necroză.

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# LIFE QUALITY IN HEALTH VISION

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**Abstract.** Generally, the context in which an individual exist is of great importance for his health status and the quality of his life status. Understanding the nature and cause of disease provides a basis for preventive action and control as even to improve the life quality. Health is not only a fundamental determinant of both the length and the quality of people's lives and it must mostly be defined in relation to the environmental and human characteristics. Some lifestyle choices as: smoking cigarettes, poor diet, inactivity, lack of sleep, excessive alcohol consumption, neglect of oral hygiene, contribute as important factors to poor health. As a result, among the current health problems today, are the prevention and control of conditions who conduct to several illnesses appearance, like: cancer, diabetes mellitus, arthritis, musculoskeletal diseases, and the mental changes associated with aging. Health education and its promotion programs generally are focused on the health and well-being of individuals. In order for health terms, the language used by health professionals in their programs, has to be a normal one that others who are not in the medical field can understand. All of the above health problems can be prevented or significantly reduced through effective school health activity.

**Key words:** life quality, health promotion, health education.

## Life quality

The quality of life includes not only wealth and employment but also the built environment, physical and mental health, education, recreation and leisure time, and social belonging (Deac, 2016). Within the field of healthcare, quality of life is often regarded in terms of how a certain ailment affects a patient on an individual level (WHO, 1984). Women may live longer than men in the EU, but men spend a comparatively greater part of their lives in good health. A healthy condition is defined as one without limitations in daily functioning (Kirckbusch, 1986). The general improvement in health conditions in Europe does not mean that the European Union does not face potential threats to its future health situation. For example, smoking and obesity, among the main causes of premature deaths, are regarded as major threats to public and individual health. Overall, Europeans enjoy near-universal access to healthcare. Their life expectancy is already among the highest in the world and is fast increasing and infant mortality rates have dropped to impressively low levels (Adams et al., 2006).

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Some variations persist from one country to another, but health outcomes indicators in eastern European countries which joined the EU in 2004 and 2007 are also improving today.

**Health meaning**

Health is not only a value in itself. Today health is defined in relation to the environmental and human characteristics of people's daily lives and the links between them. Health includes the impact of human activities on the health of individuals and groups, their economy and their environment (Deac, 2010). Hunger, malnutrition, malaria, water-borne diseases, drug and alcohol abuse, violence and injury, unplanned pregnancy, HIV and AIDS and other sexually transmitted infections are just some of the problems that have enormous implications for health. Generally, the context in which an individual lives is of great importance for both his health status and quality of their life (Frohlich et al., 2001). There are a lot of types of health issues common with many people across the globe.

Some contributing factors to poor health are lifestyle choices. These include: smoking cigarettes, poor diet, inactivity, lack of sleep, excessive alcohol consumption, neglect of oral hygiene. There are also genetic disorders that are inherited by the person and can vary in how much they affect the person and when they surface. The first public health revolution addressed sanitary conditions and fought infectious diseases; the second public health revolution focused on the contribution of individual behaviors to noncommunicable diseases and premature death. "What makes people healthy?" is not a simple question to be answered today and it will need to address both the collective lifestyles of modern societies and the social environments of modern life as they affect the health and quality of life of populations (Simmons-Morton et al., 2005).

**Health promotion**

Health promotion is defined by WHO: "the process of enabling people to increase control over and to improve their health" (Ottawa Charter for Health Promotion, 1986). Health promotion is focused on physical, mental and social well-being not merely disease or infirmity in line with the definition of health (WHO, 1984). The most important avenue for the spread and recognition of a broad understanding of health promotion was the adoption of 38 Health for All targets by the member states of the European region (Mai et al., 2005). Health promotion represents the mediation strategy between people and their environment (ecosystem) that synthesizes personal choice and the responsibility of the society towards health. Public health action depends not only on governmental organization and public attitudes but also on the health sciences and their technology. Understanding the nature and cause of disease provides a basis for preventive action and control. As a result, among the current problems are the prevention or control of chronic conditions such as: cancer, diabetes

mellitus, arthritis, musculoskeletal diseases, and the mental changes associated with aging (Donatelle, 2009). Health promotion is the process of enabling people to increase control over and improve their health. Policy, management and systems should provide guidance, oversight, coordination, monitoring and evaluation to ensure an effective, sustainable, and institutionalized educational response to health challenges (Deac, 2014).

**Behavior studies**

These are necessary to understand the predisposition of individuals towards certain risks factors and should be the basis for developing health promotion and education interventions (Christakis and Fowler, 2007). It will be presented next here, the most frequent known risk factors for some diseases.

***An unhealthy diet*** is a risk factor for several diseases. The risks presented by unhealthy diets start in childhood and build up throughout life. Unhealthy diets are associated with overweight and obesity, conditions that have increased rapidly in children around the world over recent years. Evidence on the extent, nature and effects of food marketing to children shows that advertising is extensive and other forms of food marketing to children are widespread across the world. Eating well is important for all of us. At the beginning of the 21st century, for the first time in human history, more of the earth's population suffers from too much food, rather than from lack of food. Making healthier dietary choices on a regular basis also allows a person to relax and enjoy their food, instead of worrying about calories and portion sizes (Deac, 2014). The increasing number of persons, who are obese, is a problem even amongst children now. In general, for a normal healthy adult to maintain body weight with just activities of daily living, caloric intake must be limited to 22 calories per kg of weight. Of course, growing children require more calories. Young adults can generally eat more and not gain weight. Food intake is regulated via neural circuits located in the hypothalamus. Social factors play a major role in weight gain. Situations during life in which weight gain is more likely to occur include: adolescence, pregnancy, mid-life in women, and following marriage in men. Persons who emigrate to a more urbanized culture tend to gain weight. Weight gained during holiday periods and festivals is more than at other times of the year. Increasing the complexity of carbohydrates that are consumed on a regular basis and reducing the simple sugars in the diet tends to increase the stability of blood sugar levels, lowering the likelihood of developing diabetes, or other diseases. Not only the risks of heart disease, cancer, diabetes and other illnesses reduced, must be under control but also to look and feel better. Making healthier dietary choices on a regular basis also allows a person to relax and enjoy their food, instead of worrying about calories and portion sizes. But what exactly is a healthy, balanced diet, must be correct learned out by each of us (Sacks et al., 2009).

An ideal ***body mass index*** (BMI) is also very important for our health condition. In the range of 20 to 24 and anything above or below that range will

increase certain risks for morbidity and mortality. Generally, a BMI >28 increases the risk for morbidity. Using a definition of obesity as a BMI of 30 or more, makes easy to qualify a person as obese and in determination of pathology risk. The risk for higher mortality is great even for persons who are overweight (Willet et al., 1999). A central distribution of fat, as is more typical of men, carries a higher risk for morbidity. A more peripheral distribution, as in hips and thighs in women, carries a lesser risk. The risk can be determined by measuring waist circumference and by calculating a waist-to-hip circumference ratio. In general, a waist: hip circumference ratio >0.9 for men and >1.0 for women carries an increased risk for morbidity.

***Physical inactivity*** is a high-risk factor today for diseases worldwide. Globally, it is estimated to cause: diabetes, ischemic heart disease and breast and colon cancer. Recent data show that approximately 31% of the world's population does not undertake the recommended amount of physical activity to gain protective health benefits (Deac, 2014). Several environmental factors which are linked to urbanization can discourage people from becoming more active, such as: fear of violence and crime in outdoor areas; high-density traffic; low air quality, pollution; lack of parks, sidewalks and sports/recreation facilities. Physical activity helps maintain a healthy weight and has a positive influence on mental health and well-being. It is also fundamental in achieving energy balance and weight control (Deac, 2016).

***Alcohol use*** is recognized as a high risk for many early death cases. Worldwide 5% of all deaths of young people between the ages of 15 and 29, are attributable to alcohol use (Deac, 2010).

**Health education**

Health education means any combination of learning experiences designed to help individuals and communities improve their health, by increasing their knowledge or influencing their attitudes. WHO promotes school health, as a strategic means to prevent important health risks among youth and to engage the education sector in efforts to change the educational, social, economic and political conditions that affect risk (WHO, 1984). All of the above health problems can be prevented or significantly reduced through effective school health, health education and several good health programs today. Health education and promotion programs generally focus on the health and well-being of individuals, instead of addressing theory or policy (Deac, 2010). The fields of study that serve as the foundation for health promotion and education include: Biology; Health communication; Human development; Public health; Health services; Health psychology. The Joint Committee on Health Education and Promotion Terminology of 2001 defined Health Education as "any combination of planned learning experiences based on sound theories that provide individuals, groups, and communities the opportunity to acquire information and the skills needed to make quality health decisions. The World Health Organization defined

Health Education as "comprising of consciously constructed opportunities for learning involving some form of communication designed to improve health literacy, including improving knowledge, and developing life skills which are conducive to individual and community health. Education for health begins with people (McKenzie et al., 2009). Health education is also an effective tool that helps improve health in developing nations. It not only teaches prevention and basic health knowledge but also conditions ideas that re-shape everyday habits of people with unhealthy lifestyles in developing countries. This type of conditioning not only affects the immediate recipients of such education but also future generations will benefit from an improved and properly cultivated ideas about health that will eventually be ingrained with widely spread health education (Schillinger et al., 2006). Moreover, besides physical health prevention, health education can also provide more aid and help people deal healthier with situations of extreme stress, anxiety, depression or other emotional disturbances to lessen the impact of these sorts of mental and emotional constituents, which can consequently lead to detrimental physical effects. There are many countries which developed a Health Education curriculum. Since 2001, the Ministry of Education, Research, Youth and Sports developed a national curriculum on Health Education. The National Health Education Program in Romanian Schools was considered as being a priority for the intervention of the GFATM (Global Fund) and UN Agencies. In recent years the widening horizons of public health have brought to our attention problems of chronic disease, but also such problems as accident prevention, mental health, addictive diseases, the organization of medical care, and increasing needs for social services. Also important in the light of current problems is the renewed emphasis on control of the physical environment.

Many health problems have been solved basically, but knowledge awaits application in practice. In all countries there are problems of community health that require political and social action guided by available knowledge. In this sense, the dynamic and changing character of community health action and the significant trends and issues involved in it must be viewed as an aspect of the process of social change in society. Health promoters and educators often work with the community. WHO promotes school health programs as a strategic means to prevent important health risks among youth and to engage the education sector in efforts to change the educational, social, economic and political conditions that affect as a real for disease risks. In order to have a population that understands health terms and can make proper health decisions, the language used by health professionals has to be a level that others who are not in the medical field can understand. That for, health professionals must know their audience in order to better serve their patient. The more health literate people are the more they are able to protect their health. All of the above health problems can be prevented or significantly reduced through effective school health and youth health by health education and several good health programs.

**Conclusions**

1. Health is a multifaceted concept and there is no single indicator that can adequately assess it on a countrywide level.

2. Health problems can be prevented or significantly reduced through effective health education programmes.

3. Awareness and education are powerful ways to drive behavior changes related to health promotion.

4. Effective school health programs can be one of the most cost effective investments, a nation can make to simultaneously improve education and health.

**Rezumat.** În general contextul în care un individ există este de mare importanţă pentru statusul sănătăţii sale, dar şi pentru cel al vieţii sale în general. Înţelegând natura şi cauza bolilor, se pot deduce aşa şi acţiunile preventive şi de control ale lor, dar şi cum să îmbunătăţim calitatea vieţii în sine. Sănătatea nu este doar o determinantă fundamentală a lungimii şi calităţii vieţii populaţiei, ci trebuie mai mult definită în relaţie cu mediul înconjurător şi cu caracteristicile specifice umane. Câteva preocupări şi stiluri de viaţă ca: fumat, alimentaţia deficitară, inactivitatea, somnul insuficient, consumul excesiv de alcool, incorecta igienă orală, sunt factori importanţi care conduc la o sănătate proastă. Ca rezultate printre problemele curente de sănătate de azi se numără prevenirea şi controlul condiţiilor care duc la apariţia a numeroase boli: cancer, diabet, artrite, boli musculo-scheletale şi deficienţe mintale asociate cu îmbătrânirea. Educaţia pentru sănătate şi programele de promovare sunt măsuri focusate pentru o sănătate şi stare de bine a indivizilor. Pentru termenii de sănătate, cei folosiţi de profesioniştii din sănătate în limbajul instituit al programelor lor, ei trebuie să fie simpli, aşa încât cei care nu sunt din branşa medicală să-i poată înţelege şi ei. Toate aceste aspecte din problematica de sănătate se pot reduce şi la activităţile efectuate util în şcolile de sănătate publică.

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# DEVELOPEMENT OF VACCINE AND VACCINATION

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**Abstract.** Vaccination includes various ways of administering immunogens. Vaccines can prevent or ameliorate infectious disease. Edward Jenner is considered the founder of vaccinology. It is figured out that when a sufficiently large percentage of a population has been vaccinated, herd immunity results. Vaccines generate immunity across the body as a whole, but they can also provoke specific immune responses in specific bodily areas. The revolution of genetic engineering toward the end of the 20th century has greatly impacted vaccine development. Understanding the nature and cause of disease provides a basis for preventive action and control as even to improve the life quality by using the vaccination in the world.

**Key words:** vaccines, vaccination, immunity.

## General consideration about Vaccination and Vaccines

**Vaccination** is the administration of antigenic material – a vaccine, for to stimulate an individual's immune system to develop adaptive immunity to a pathogen. In common speech, *vaccination* and *immunization* have a similar meaning. This distinguishes it from inoculation, which uses unweakened live pathogens, although in common usage either can refer to an immunization. Stimulating immune responses with an infectious agent is known as immunization (Artenstein, 2010). Vaccination includes various ways of administering immunogens. It is known that the process of inoculation was used by Chinese physicians in the 10th century. The first rabies immunization was given by Louis Pasteur to a child after he was bitten by a rabid dog (Plotkin, 2011). While vaccination provides a lasting effect, it usually takes several weeks to develop, while passive immunity, which means the transfer of antibodies, has immediate effect.

**Vaccines** can prevent or ameliorate infectious disease. When a sufficiently large percentage of a population has been vaccinated, herd immunity results. The effectiveness of vaccination has been widely studied and verified. Most vaccines are given by hypodermic injection as they are not absorbed reliably through the intestines. Live attenuated polio, some typhoid, and some cholera vaccines are given orally to produce immunity in the bowel.

Vaccines generate immunity across the body as a whole, but they can also provoke specific immune responses in specific bodily areas.

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For this reason, the varying delivery methods of vaccines are important. However, specific delivery routes are also sometimes necessary to minimize the chances of vaccines having adverse effects on the body (Hutin et al., 2003). Depending on the vaccine(s) that are to be administered, and the age and size of the person to be vaccinated, decide on the appropriate injection site and route, and the injection equipment required (*Fig. 1*).

**Fig. 1.** Vaccine aspect (Plotkin, 2011)

Most vaccines available in the world are given intramuscularly. Only a few vaccines are given subcutaneously, orally or intradermally. It is important that infants and children do not move during injection of vaccines. Most vaccines can be administered into the deltoid area. The choice of injection site depends on the age of the person to be vaccinated.

**Practice of vaccination**

According to the World Health Organization, 1,500 people die of an infectious disease every hour. Vaccines are given to people when they are at risk of contracting a disease. Many vaccines are given at a young age because children's bodies may not be strong enough to fight off naturally occurring diseases, which puts them at risk. The practice of immunization dates back hundreds of years. According to the literature, the history of vaccination can be traced back to as early as the 7th century when the monks in India tried to immunize themselves by drinking snake venom. The first vaccination was inoculation with human smallpox, a practice widely carried out in ancient India, Arabia, and China.

Edward Jenner is considered the founder of vaccinology in the West in 1796, after he inoculated a 13-year-old boy with vaccinia virus (cowpox), and demonstrated immunity to smallpox (Baxby, 2011 in Plotkin, 2011). In 1798, the first smallpox vaccine was developed. Over the 18th and 19th centuries, systematic implementation of mass smallpox immunization culminated in its global eradication in 1979. Smallpox became a preventable disease by injecting pus extracted from a human infected with cowpox virus. Jenner named the

substance "vaccine" after the Latin word "vacca" which means "cow", and thus the process of giving vaccine became "vaccination". The story of vaccines did not begin with the first vaccine–Edward Jenner's use of material from cowpox pustules to provide protection against smallpox.

Vaccination spread across the globe – although these early vaccines were crude, they worked. The first vaccination programmes dramatically reduced the number of deaths from disease and were crucial in establishing the concept of preventative public health measures. Rather, it begins with the long history of infectious disease in humans, and in particular, with early uses of smallpox material to provide immunity to that disease.

The small pox vaccination efforts had historical significance because ability to control the infectious disease was achieved and the vaccination saved many lives by preventing smallpox during this period. Edward Jenner, Louis Pasteur, and Maurice Hilleman, pioneers in vaccine development receive particular attention as well. Maurice Hilleman was the most prolific vaccine inventor, developing successful vaccines for measles, mumps, hepatitis A, hepatitis B, chickenpox, meningitis, pneumonia and Haemophilus influenzae.

World War II accelerated vaccine development. Fear of a repetition of the 1918–19 world epidemic of influenza focused urgent attention on all viral diseases, while commercial production of antibiotics taught researchers to grow viruses with less microbe contamination. Also, investigators paid closer attention to vaccine safety and effectiveness through clinical studies before release of a vaccine to the public, especially after the yellow fever vaccine apparently caused hepatitis B in many U.S. soldiers in 1942.

In 1948, Bacillus Calmette-Guérin (BCG) vaccine was produced and inoculated, and in 1949, the Central Quarantine Laboratory produced anti-serum and performed vaccination against 18 preventable diseases. In March 1948, John Enders, Thomas Weller, and Frederick Robbins used human embryonic skin and muscle tissue, grown in a nutrient mix with antibiotics, to prove poliovirus could infect tissue other than nerve cells. Their confirmation meant that researchers could now grow enough poliovirus to create large quantities of vaccine. The three scientists won the Nobel Prize in Physiology or Medicine in 1954, the year polio vaccine had its first large clinical trial. Neither Jonas Salk nor Albert Sabin received a Nobel Prize for their work in creating vaccines (*Fig. 2*).

In 1954, the Infectious Disease Prevention Act was established and routine vaccination was specified. Smallpox, diphtheria, whooping cough, typhoid fever, typhus, paratyphoid fever, and tuberculosis vaccination were given. Diphtheria-tetanus-pertussis (whooping cough). DTP vaccine in 1955, killed polio vaccine in 1958, and inactivated vaccine for typhoid fever in 1960 were used.

**Fig. 2.** Jonas Salk's and Albert Sabin's glory effect (Plotkin, 2011)

In 1991, the Pan American Health Organization located the last wild-type polio case in the Western Hemisphere-a nine-year-old boy in Peru. The last person to contract wild-polio in the western Pacific region occurred in 1997, and in Europe in 1998. In developing nations, the eradication of polio and attempts to control HIV/AIDS and malaria are in competition for funds and workers. India reported only eight new polio cases in 2004; Nigeria reported 257. By comparison, a total of about four million people are living with HIV/AIDS in India and Nigeria. A high success appeared for the end of Polio after using in USA the vaccination (*Fig. 3*).


**Fig. 3.** End of Polio in newspapers (Plotkin, 2011)

To eliminate the risk of outbreaks of some diseases, at various times governments and other institutions have employed policies requiring vaccination for all people. Beginning with early vaccination in the nineteenth century, these policies were resisted by a variety of groups, collectively called antivaccinationists, who object on scientific, ethical, political, medical safety,

religious, and other grounds. Although vaccination was taken up enthusiastically by many, there was some violent opposition as it became more widespread.

In countries with limited financial resources, limited vaccination coverage results in greater morbidity and mortality due to infectious disease. More affluent countries are able to subsidize vaccinations for at-risk groups, resulting in more comprehensive and effective coverage. The World Health Organization (WHO) estimate that vaccination averts 2-3 million deaths per year (in all age groups), and up to 1.5 million children die each year due to diseases which could have been prevented by vaccination.

Proper vaccine administration is critical to ensure that vaccination is safe and effective. CDC recommends that all health care personnel who administer vaccines receive comprehensive, competency-based training on vaccine administration policies and procedures before administering vaccines. Patients and their family members count on health care personnel to administer vaccines safely. Always screen patients for contraindications and precautions before a vaccine is administered, even if the same vaccine was administered previously. Each vaccine has a recommended administration route and site. This information is included in the manufacturer's package insert for each vaccine. If multiple vaccines are administered at a single visit, administer each injection in a different injection site.

The criteria for selection of the national immunization program is effectiveness, stability, cost-benefit, convenient application, financial resources, storage and social-cultural standards. It specifies coverage of vaccination, quality and standard methods of vaccination, detailed policies and eradication of preventable infectious diseases. The Romanian criteria allow some important one, as *Fig. 4* shows out.

The middle of the 20$^{th}$ century was an active time for vaccine research and development. Methods for growing viruses in the laboratory led to rapid discoveries and innovations, including the creation of vaccines for polio. Researchers targeted other common childhood diseases such as measles, mumps, and rubella, and vaccines for these diseases reduced the disease burden greatly.

It is obvious that from the late 19th century, vaccines could be developed in the laboratory. However, in the 20th century, it became possible to develop vaccines based on immunologic markers. In the 21st century, molecular biology permits vaccine development that was not possible before. The revolution of genetic engineering toward the end of the 20th century has greatly impacted vaccine development.

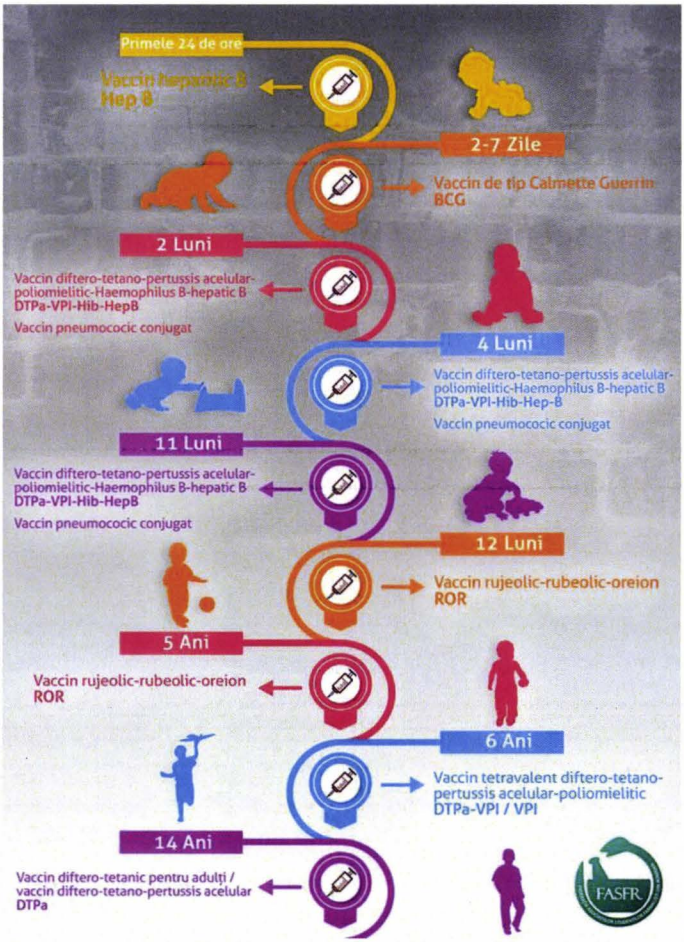


**Fig. 4.** Immunization in Romania (from newborn to age14) – Programul Național de Imunizare al MS din Ro

**New orientation in the world vaccination activities**

The past two decades have seen the application of molecular genetics and its increased insights into immunology, microbiology and genomics applied to vaccinology. Current successes include the development of recombinant hepatitis B vaccines, the less reactogenic acellular pertussis vaccine, and new techniques for seasonal influenza vaccine manufacture.

Molecular genetics sets the scene for a bright future for vaccinology, including the development of new vaccine delivery systems (e.g. DNA vaccines, viral vectors, plant vaccines and topical formulations), new adjuvants, the development of more effective tuberculosis vaccines, and vaccines against cytomegalovirus (CMV), herpes simplex virus (HSV), respiratory syncytial virus (RSV), staphylococcal disease, streptococcal disease, pandemic influenza, shigella, HIV and schistosomiasis among others (Huber, 2014). Innovative

techniques now drive vaccine research, with recombinant DNA technology and new delivery techniques leading scientists in new directions. Disease targets have expanded, and some vaccine research is beginning to focus on non-infectious conditions such as addiction and allergies. Therapeutic vaccines may also soon be available for allergies, autoimmune diseases and addictions.

**Conclusions**

The growing interest of public health related to vaccination reflects that they are well aware of preventable diseases and safety issues of vaccines unlike in the past.

The development of vaccination has been highlighted as a striking achievement of the modern medical sciences with new technologies in many fields of medicine.

With spontaneous introductions of new vaccines, the selection of vaccines to be produced and to be used must be determined based on local disease epidemiology and disease burden of each and every applied region.

Researches in many areas are under way and we hope that some of them may be preventable and decreased with a development of new vaccines in the future.

Vaccination is a miracle of modern medicine.

**Rezumat.** Vaccinarea include multiple căi de administrare a imunogenilor. Vaccinurile pot preveni sau ameliora apariția bolilor infecțioase. Edward Jenner este considerat a fi inventatorul vaccinologiei. S-a dovedit că dacă un procent mare de populație este vaccinat se poate conta și pe rezultate imunologice bune. Vaccinurile generează imunitatea în corp, dar pot face și imunitate specifică în unele areale specifice ale organismului. Dezvoltarea ingineriei genetice de la sfârșitul secolului XX a însemnat și prezența unei dezvoltări considerabile a vaccinologiei. Înțelegând natura îmbolnăvirilor, putem determina acțiuni de prevenire și control utile, dar putem și să îmbunătățim calitatea vieții prin folosirea vaccinării în întreaga lume.

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# AWARENESS FOR PARENTS OF CHILDREN IN AN ANIMAL ASSISTED THERAPY (TAA) PROGRAM

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**Abstract.** Autism spectrum disorders are becoming more prevalent among children. Early diagnosis of behavioral disorders and early treatment are essential for the harmonious development of the affected children. One of the newest methods of therapy, the Animal Assisted Therapy (TAA) promotes positive human-animal interaction by incorporating the psychological, behavioral and physical features of an animal into a therapeutic environment, in order to facilitate the patient's recovery process.

**Key words:** animal assisted therapy, childhood disorder, autism, physical-recovery.

## Introduction

Developmental neuropsychiatric disorder is considered one of the most severe childhood disorders, autism, is alarmingly growing worldwide reaching an incidence of 1:100 affected children, according to a study conducted in 2012 (Mareş et al., 2015).

The symptoms are extremely varied and can change within the same subject over time. However, early diagnosis (up to 3 years of age) and early treatment are essential for the development of affected children to their full potential.

Among the most accessible and used treatment methods are those based on improving communication, social aspects, adaptive behavior and the child's learning ability through a series of structured and specialized treatments that have the role of reducing the characteristic symptoms and support the child's development.

The most common forms of treatment are the occupational therapy and physiotherapy, speech therapy and drug treatments aimed to treating behavioral problems and depression, anxiety, hyperactivity and obsessive-compulsive behavior. In the case of preschoolers, a high success rate has been shown to have the structured educational therapies that involve strengthening social skills, communication and behavior.

In the last thirty years, special attention has been paid to activities based on human-animal interactions, in educational therapeutic programs addressed to people diagnosed with autism, institutionalized elderly people, in the phase of physical recovery, people with depressive symptoms, etc. (Rusu, 2017).

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Thus, TAA (Animal Assisted Therapy) has become a successful therapeutic model that involves an animal in the therapeutic environment in order to improve the quality of human life, to facilitate the recovery process of people in need of physical or mental care (Chandler, 2005).

In this respect, animal-assisted activities (AAA) also play a particularly important role, which can provide motivational, educational and/or recreational opportunities and enrich the quality of life, as a result of the positive human-animal interaction, regardless of whether it is a pet, a therapy animal or animals from the natural environment or captivity (farms, gardens, zoos, etc.) (Bonas et al., 2000; Myers, 2000).

**Objectives**

Being models of therapy with extremely wide applicability (children, adults, seniors, people with disabilities, depressed people, people in a recovery therapy, people suffering from anxiety, social isolation, psycho-motor disorders, etc.) animal-assisted activities become an extremely dynamic field with possibilities for large-scale implementation (kindergartens, schools, hospitals, recovery centers, etc.), based on a two-way voluntary relationship with bilateral or multilateral benefits (Katcher, 2000).

Regarding the introduction of these methods of therapy/animal-assisted activities in a program for preschoolers, a key element is the awareness of direct and indirect beneficiaries of both, the benefits and the possible risks involved in these human-animal interactions.

In this respect, the realization of an information/awareness program will be considered, which will have as main objectives:

- establishing the scope of activities (cognitive, behavioral, emotional, psychosocial, language psychotherapy, etc.) (Fine, 2000; Chandler, 2005);
- developing a positive therapeutic relationship;
- increasing the motivation of the patient (s) to participate in therapy sessions or the proposed activities;
- stimulating the positive experiences and facilitating the achievement of therapeutic goals or results, which would be more difficult to achieve without the assistance of the animal;
- increasing the level of trust in the therapy and in the therapist;
- maximizing the efficiency of therapy sessions by drawing attention from the discomfort associated with a particular condition to the joy and comfort associated with interacting with the animal;
- reducing aggressive behaviors in preschool children, or identifying aggressive behaviors;
- Understanding by parents the benefits brought by such activities and their involvement in the program of activities;

- knowledge of the risks involved in such activities (bites, scratches, allergies, blows, zoonoses) by both parents and educators, or support staff who may be involved in the program;
- awareness of the risks of contacting zoonotic diseases and the necessary measures to prevent them;
- identification of attitudinal and behavioral factors associated with the optimal interaction between humans and animals;
- awareness of the socio-emotional, affective benefits of human-animal interactions;
- increasing self-esteem, responsible attitudes, by raising awareness of the animal's needs;
- Improving children's emotional intelligence;
- increasing the motivation and interest of children for the activities carried out;
- educating parents and children for the responsible possession of an animal;
- overcoming anxiety, phobia, emotional discomfort, social integration etc.

**Methodology**

According with the chosen program, the applicability methods must be extremely varied to cover and satisfy the whole range of needs both in terms of the animal or animals involved in the activities and the needs and necessities of the beneficiaries.

In this regard, the program should start with a program presentation campaign specifying the purpose, the objectives pursued and last but not least, the expected results.

In order to familiarize all the partners involved (parents, educators, psychologists, psychotherapists, speech therapists) the program requires a training in which are presented the benefits of children's interaction with an animal, but also the risks of such interaction, respectively, awareness of all methods which are the basis for the prevention of zoonotic infections, or abusive behaviors on animals.

It is important to familiarize parents with the methods used in such animal-assisted activities and to inform them about the applicability and results obtained so far of such programs.

Conducting sessions of debates or meetings with beneficiaries or specialists in the field to provide the best answers.

Presentation of some activities carried out and results obtained. The necesities of parents contact with the animal involved in the activity, with the therapist or program coordinator.

During the program, the permanent monitoring of the children within the program, the preparation of reports in order to record the progress, or the changes occurred during the beneficiaries' interaction with the animal will be taken into account.

The results obtained will be interpreted and communicated personally and generally to all beneficiaries involved in the program. It will be considered to highlight the strengths and weaknesses, the benefits observed, the improvement needed, the plan of measures to be taken to maximize the results obtained.

**Expected results**

In terms of expected results, it is clear that each program is based on previous experiences. As animal-assisted activities are increasingly accepted and accessed by specialists in various fields, due to the alternative clinical strategies proposed to treat or ameliorate mental, emotional, behavioral or even physical-recovery disorders, the expected results are aimed at certainly obtaining some benefits in terms of maintaining an optimal mental and emotional comfort, by satisfying the social, physical, motor needs, of exploring the environment, and of the affective-emotional behaviors.

Among the expected positive results are:

- Increasing parents' interest in new animal-assisted therapy techniques and stimulating confidence in accepting these activities as an integral part in the recovery process of preschool/ school children in a different setting (kindergarten, school) and seen as part of the educational act not strictly therapeutic, which would considerably reduce the child's negative, anxious experiences in contact with the therapist, the educator, making the hours or sessions desired, expected by the child in terms of meeting and interacting with the animal. The animal has a role in inducing and maintaining the well-being, joy of the child, to induce self-confidence, self-control, mastery, desire for collaboration, in a natural and pleasant way.
- Decreased level of stress, anxiety, depression, feelings of loneliness, vulnerability;
- Improving attentional skills, increasing the level of autonomy;
- Increasing the degree of empathy and the degree of communication, relationships and socialization;
- Involvement of parents, community in programs, projects for disseminating experiences, and the results obtained through activities based on human-animal interactions;
- Empowering adults and children through examples of good practice (an abandoned animal used in such activities can be an example of rehabilitation, which can lead to the prevention of negative attitudes, abandonment of animals, or encouraging adoptions and responsible ownership of animals);
- Promoting positive attitudes, respect for nature and living beings;
- Promoting and increasing the number of volunteers involved in such of projects.

There is also the possibility that the results obtained may not always be positive but may be negative, and this may result from the dynamics of the

emotional states of the people and animals used in the program. During the work sessions there may be a number of disturbing factors that affect the ability to maintain the attention of the child or animal, and the expected response may be different from the expected one (Rusu, 2017).

A negative aspect is the impossibility of involving in activities people with allergies to animals, or increased phobias towards animals, or people with perceptual disorders (they feel that the animal rejects them, which can accentuate the decrease in self-esteem) or of cases in which the animal is at risk of injury due to improper interaction (Stanciu, 2016).

There is a risk of separation discomfort at the end of the activity when the child has to separate from the animal. Some parents may have a negative or distrustful attitude in accepting these activities, may have different, suspicious opinions and it is very important to make it known that these activities are a therapeutic alternative and as with any other therapeutic method can not be guaranteed the result. It is especially important to make parents aware of every moment / progress, even a minimal one, recorded during the work sessions.

**Rezumat.** Tulburările din spectrul autismului sunt din ce în ce mai răspândite în rândul copiilor. Diagnosticarea precoce a tulburărilor de comportament şi tratamentul timpuriu sunt esenţiale pentru dezvoltarea armonioasă a copiilor afectaţi. Una dintre cele mai noi metode de terapie, Terapia asistată de animale (TAA) promovează interacţiunea pozitivă între om şi animal prin încorporarea trăsăturilor psihologice, comportamentale şi fizice ale unui animal într-un mediu terapeutic, cu scopul de a facilita procesul de recuperare al pacientului.

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ISSN 2069 - 1521