



ANDRIJA STAMPAR
TEACHING INSTITUTE
OF PUBLIC HEALTH

Creating a Healthier Future

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in Current Contents

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FOREWORD

The *2018 Review of Professional and Scientific Papers* is an annual report on professional and scientific papers by the employees of the Teaching Institute published in various professional and scientific journals and books, or presented at conferences and events, in the course of 2018. The papers in the Review are classified according to *Hrvatska znanstvena bibliografija* – CROSBİ (Croatian Scientific Bibliography).

The Croatian version of the 2018 Review includes a record 195 abstracts (there were 164 abstracts in 2017 and 179 in 2016), with 18 of them in *Current Contents* journals (20 such papers in 2017 and 17 in 2016). The English version brings the mentioned 18 abstracts of original scientific papers and reviews published in *Current Contents*-journals.

The year 2018 was also marked by the launch of the “Food Safety and Quality Centre” program at the Teaching Institute. The sixth component of the project is scientific-research work.

There were 54 scientists working at the Teaching Institute in 2018: 38 with doctoral degrees and 16 with master’s degrees. The percentage of female scientists dominates (four in five) the overall number: 44 female scientists versus 10 male scientists.

The 2018 Review reflects the multidisciplinary, diversity, and productivity of the experts employed at our institution who are, in addition to their primary positions, engaged in professional and scientific work. The number of abstracts in the 2018 Review reveals that 2018 was successful for the Teaching Institute in terms of professional and scientific work, and we congratulate the employees of the Institute and their external associates for their every contribution.

Institute Head

Zvonimir Šostar, MD

ORIGINAL SCIENTIFIC AND
REVIEW ARTICLES IN
CURRENT CONTENTS

1. AN OUTBREAK OF HAEMORRHAGIC FEVER WITH RENAL SYNDROME LINKED WITH MOUNTAIN RECREATIONAL ACTIVITIES IN ZAGREB, CROATIA, 2017

Epidemiology and Infection. 2018;146(10):1236-1239

Impact Factor: 2.044

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Abstract

In 2017 Zagreb faced the largest outbreak of haemorrhagic fever with renal syndrome (HFRS) to date. We investigated to describe the extent of the outbreak and identify risk factors for infection. We compared laboratory-confirmed cases of Hantavirus infection in Zagreb residents with the onset of illness after 1 January 2017, with individually matched controls from the same household or neighbourhood. We calculated adjusted matched odds ratios (amOR) using conditional logistic regression.

During 2017, 104 cases were reported: 11-81 years old (median 37) and 71% (73) male. Compared with 104 controls, cases were more likely to report visiting Mount Medvednica (amOR 60, 95% CI 6-597), visiting a forest (amOR 46, 95% CI 4.7-450) and observing rodents (amOR 20, 95% CI 2.6-159). Seventy per cent of cases (73/104) had visited Mount Medvednica prior to infection. Among participants who had visited Mount Medvednica, cases were more likely to have drunk water from a spring (amOR 22, 95% CI 1.9-265), observed rodents (amOR 17, 95% CI 2-144), picked flowers (amOR 15, 95% CI 1.2-182) or cycled (amOR 14, 95% CI 1.6-135). Our study indicated that recreational activity around Mount Medvednica was associated with HFRS. We recommend enhanced surveillance of the recreational areas during an outbreak.

Keywords: Croatia, epidemics, hantavirus, zoonoses

2. ANALYSIS OF HEALTH-RELATED BIOMARKERS BETWEEN VEGETARIANS AND NON-VEGETARIANS: A MULTI-BIOMARKER APPROACH

Journal of Functional Foods. 2018;48:643-653

Impact Factor: 3.470

Gajski G¹, Gerić M¹, Vučić Lovrenčić M², Božičević S², Rubelj I³, Nanić L³, Škrobot Vidaček N³, Bendix L⁴, Peraica M⁵, Rašić D¹, Domijan AM⁵, Gluščić V¹, Jurasović J¹, Orct T¹, Cvijetić Avdagić S¹, Jurak G⁶, Bošnjir J⁶, Garaj Vrhovac V¹

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Abstract

This study was performed in a group of adult vegetarians (N = 40) and matched non-vegetarian subjects (N = 40) in order to analyse differences in health-related biomarkers. Obtained results revealed differences in various biomarkers between subjects on a traditional mixed and vegetarian diet, indicating that vegetarians have a lower nutritional status of some nutrients (Ca, Cu and Zn, and vitamins B₁₂ and D) accompanied with a lower antioxidant defence system (glutathione) and higher homocysteine and genome damage (micronuclei and DNA strand breaks), along with shorter telomeres. This suggests that the supplementation of animal derived nutrients to this particular dietary group would be beneficial for the improvement of some measured health-related biomarkers. However, the level of certain toxic metals (As and Hg) was higher in

non-vegetarians. The presented multi-biomarker approach implies the necessity of evaluating a large number of different health-related biomarkers in order to obtain clear insight into dietary preferences and health outcomes.

Keywords: vegetarians, non-vegetarians, dietary habits, oxidative stress, genomic instability, health-related biomarkers

3. ATTITUDES AND BELIEFS RELATED TO CHILDHOOD VACCINATIONS AMONG PARENTS OF 6 YEARS OLD CHILDREN IN ZAGREB, CROATIA

Vaccine. 2018;36(49):7530-7535

Impact Factor: 3.285

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Abstract

The Croatian Immunization Programme is a mandatory prevention programme in which vaccines are offered free of charge and little is known about parental vaccine-related beliefs and attitudes. We aimed to describe beliefs and attitudes towards childhood vaccination of parents in Zagreb, estimate the proportion of parents with positive attitudes towards vaccination, and to identify the possible predictors of positive attitudes. Using a self-administered questionnaire, we performed a cluster sampling survey among parents of 6-years-old children in Zagreb. In total 542 questionnaires were collected, 80% (n = 430/542) of respondents were mothers. Even though 72.6% (n = 385/531; 95% CI: 69–76) respondents feel that childhood vaccination should remain mandatory, 36.3% (n = 192/528; 95% CI: 58.3–65.3) considered that simultaneous administration of vaccines can have negative effect to their child's health. In addition, 38% (n = 202/532; 95% CI: 33.2–43.1%) feared that vaccines may harm their child. Of total, parents mostly reported positive attitude towards vaccination (61.8%; 95% CI: 34.7–42).

Parents with positive attitudes were more likely to state their child experienced mild or no adverse reaction after vaccination, report not delaying vaccination and provide additional non-mandatory vaccines to their child ($p < 0.05$). Gender, age, education and marital status of parent were not significantly associated with the positive attitude towards vaccination.

Our results suggest the need for educational interventions and communication strategies that could foster better knowledge on immunization with a focus on parental misconceptions, perceived constraints and safety issues about vaccine.

Keywords: vaccination, immunization, attitude, parents, children, Croatia

4. BURDEN OF *STREPTOCOCCUS PNEUMONIAE* AND *HAEMOPHILUS INFLUENZAE* TYPE B DISEASE IN CHILDREN IN THE ERA OF CONJUGATE VACCINES: GLOBAL, REGIONAL, AND NATIONAL ESTIMATES FOR 2000-15

Lancet Global Health. 2018;6(7):e744-e757

Impact Factor: 17.686

Wahl B¹, O'Brien KL¹, Greenbaum A¹, Majumder A¹, Liu L², Chu Y², Lukšić I³, Nair H^{4,5}, McAllister DA⁶, Campbell H⁴, Rudan I⁴, Black R², Knoll MD¹

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Abstract

Background: Pneumococcal conjugate vaccine (PCV) and *Haemophilus influenzae* type b (Hib) vaccine are now used in most countries. To monitor global and regional progress towards improving child health and to inform national policies for disease prevention and treatment, we prepared global, regional, and national disease burden estimates for these pathogens in children from 2000 to 2015.

Methods: Using WHO and Maternal and Child Epidemiology Estimation collaboration country-specific estimates of pneumonia and meningitis mortality

and pneumonia morbidity from 2000 to 2015, we applied pneumococcal and Hib cause-specific proportions to estimate pathogen-specific deaths and cases. Summary estimates of the proportion of pneumonia deaths and cases attributable to these pathogens were derived from four Hib vaccine and six PCV efficacy and effectiveness study values. The proportion of meningitis deaths due to each pathogen was derived from bacterial meningitis aetiology and adjusted pathogen-specific meningitis case-fatality data.

Pneumococcal and Hib meningitis cases were inferred from modelled pathogen-specific meningitis deaths and literature-derived case-fatality estimates. Cases of pneumococcal and Hib syndromes other than pneumonia and meningitis were estimated using the ratio of pathogen-specific non-pneumonia, non-meningitis cases to pathogen-specific meningitis cases from the literature. We accounted for annual HIV infection prevalence, access to care, and vaccine use.

Findings: We estimated that there were 294,000 pneumococcal deaths (uncertainty range [UR] 192,000-366,000) and 29,500 Hib deaths (18,400-40,700) in HIV-uninfected children aged 1-59 months in 2015. An additional 23,300 deaths (15,300-28,700) associated with pneumococcus and fewer than 1,000 deaths associated Hib were estimated to have occurred in children infected with HIV. We estimate that pneumococcal deaths declined by 51% (7-74) and Hib deaths by 90% (78-96) from 2000 to 2015.

Most children who died of pneumococcus (81%) and Hib (76%) presented with pneumonia. Less conservative assumptions result in pneumococcal death estimates that could be as high as 515,000 deaths (302,000-609,000) in 2015. Approximately 50% of all pneumococcal deaths in 2015 occurred in four countries in Africa and Asia: India (68,700 deaths, UR 44,600-86,100), Nigeria (49,000 deaths, 32,400-59,000), the Democratic Republic of the Congo (14,500 deaths, 9,300-18,700), and Pakistan (14,400 deaths, 9,700-17,000). India (15,600 deaths, 9,800-21,500), Nigeria (3,600 deaths, 2,200-5,100), China (3,400 deaths, 2,300-4,600), and South Sudan (1,000 deaths, 600-1,400) had the greatest number of Hib deaths in 2015.

We estimated 3.7 million episodes (UR 2.7 million-4.3 million) of severe pneumococcus and 340,000 episodes (196,000-669,000) of severe Hib globally in children in 2015.

Interpretation: The widespread use of Hib vaccine and the recent introduction of PCV in countries with high child mortality is associated with reductions in Hib and pneumococcal cases and deaths. Uncertainties in the burden of pneumococcal disease are largely driven by the fraction of pneumonia deaths attributable to pneumococcus. Progress towards further reducing the global burden of Hib and pneumococcal disease burden will depend on the efforts of a few large countries in Africa and Asia.

Keywords: pneumococcal conjugate vaccine (PCV), *Haemophilus influenzae* type b (Hib) vaccine, child health

5. CHLORINATION OF 5-FLUOROURACIL: REACTION MECHANISM AND ECOTOXICITY ASSESSMENT OF CHLORINATED PRODUCTS

Chemosphere. 2018;207(18):612-619

Impact Factor: 4.427

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Abstract

What happens to drugs in the chlorinating environment? Degradation products may vary in pharmacological profiles and in ecotoxicity potentials compared to the parent compound. This study combines synthesis, NMR spectroscopy, quantum chemical calculations, and toxicity experiments on *Daphnia magna* to investigate chemical fate of antineoplastic drug 5-fluorouracil (5-FU) in chlorinated environment, which is common in waste-water treatment procedures, but also endogenous in activated neutrophils. A reduction of toxicity (EC₅₀ after 48 h is 50% higher than for the parent 5-FU) was observed after the first chlorination step, in which a chlorohydrin 5-chloro-5-fluoro-6-hydroxy-5, 6-dihydrouracil was formed.

Further chlorination leads to N-chlorinated intermediate, that undergoes the pyrimidine ring opening reaction. The final product, 2-chloro-2-fluoro-3, 3-dihydroxypropanoic acid was obtained after the loss of the chlorinated urea fragment.

This is the most potent compound in the reaction sequence, with toxicity parameter EC₅₀, after 48 h, more than twice lower compared to the parent 5-FU.

Clearly, the contact time between chlorinating species and degradation products provide different ecotoxicological properties of reaction mixtures. Interplay between experimental and theoretical procedures, to properly describe reaction pathways and provide more information on toxicity profiles, is a way forward in environmental science research.

Keywords: 5-fluorouracil, hypochlorous acid, ecotoxicity, reaction mechanism, DFT calculation

6. DIAGNOSTIC SIGNIFICANCE OF IMMUNOGLOBULIN G AVIDITY IN SYMPTOMATIC AND ASYMPTOMATIC WEST NILE VIRUS INFECTION

Revista da Sociedade Brasileira de Medicina Tropical. 2018;51(5):591-595

Impact Factor: 0.444

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Abstract

Introduction: West Nile virus (WNV) immunoglobulin M (IgM) antibodies have been shown to persist for up to 500 days in certain patients. To evaluate the usefulness of immunoglobulin G (IgG) avidity assessment in the diagnosis of WNV infection, we analyzed 54 WNV IgM- and/or IgG-positive serum samples from 39 patients with neuroinvasive disease and 15 asymptomatic cases tested during a seroprevalence investigation.

Methods: Serological tests (WNV IgM/IgG antibody detection, IgG avidity) were performed using commercially available enzyme-linked immunosorbent assays.

Results: WNV IgM antibodies were detected in 47 (87%) samples. Acute/recent WNV infection was confirmed based on low/borderline avidity index (AI) in 44 IgM-positive samples (93.6%). In three IgM-positive samples (6.4%), high IgG AIs were detected, thus indicating persisting IgM antibodies from previous infections. All IgM-negative samples showed high AIs. Patients with WNV neuroinvasive disease tested within 30 days showed low AIs. In six patients tested 34-50 days after disease onset, AI was borderline (42%-60%), suggesting earlier WNV IgG maturation. Samples with the highest IgM values were associated with the lowest AIs (Spearman's rho coefficient -0.767, $p < 0.001$).

Conclusions: Our results indicate that IgG avidity differentiates current/recent WNV infection from persistent IgM seropositivity from the previous WNV transmission season both in patients with WNV neuroinvasive disease and in asymptomatic persons. A strong negative correlation between IgM antibody levels and AI indicates that in cases with very high IgM levels, determination of IgG avidity may not be necessary. As many patients showed rapid avidity maturation, low IgG avidity is indicative of WNV infection within the previous month.

Keywords: West Nile virus, diagnosis, IgG avidity

7. FIRST RECORD AND SPREADING OF THE INVASIVE MOSQUITO *Aedes japonicus japonicus* (THEOBALD, 1901) IN CROATIA

Medical and Veterinary Entomology. 2019;33(1):171-176. Epub 2018 Aug 25

Impact Factor: 1.809

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Abstract

Aedes (Hulecoeteomyia) japonicus japonicus (Theobald, 1901) has recently established across North America and Central Europe. A three-year survey was conducted in northwestern Croatian regions from 2013 to 2015 using mosquito ovitraps at possible points of entry and house yards, occasionally complemented by larval collections from cemetery vases. In the first year, the survey investigated the county bordering Slovenia, where the first detection of *Ae. j. japonicus* had taken place on 28 August 2013.

During the next two years, *Ae. j. japonicus* was detected in this area from early May until late October. In 2015, several counties further to the east were

included in the survey, leading to the detection of *Ae. j. japonicus* approximately 100 km eastward from the initially surveyed region. Given a moderate continental climate and homogeneous climatic conditions in this part of Europe, the eastward spread of *Ae. j. japonicus* can be expected to continue.

Keywords: *Aedes j. japonicus*, Croatia, first record, surveillance, mosquito spreading

8. GENETIC VARIABILITY AND SEQUENCE RELATEDNESS OF MATRIX PROTEIN IN VIRUSES OF THE FAMILIES *PARAMYXOVIRIDAE* AND *PNEUMOVIRIDAE*

Intervirology. 2017;60(5):181-189. Epub 2018 Mar 6

Impact Factor: 1.011

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Abstract

Background: The families *Paramyxoviridae* and *Pneumoviridae* comprise a broad spectrum of viral pathogens that affect human health. The matrix (M) protein of these viruses has a central role in their life cycle. In line with this, molecular characteristics of the M proteins from variable viruses that circulated in Croatia were investigated.

Methods: Sequences of the M proteins of human parainfluenza virus (HPIV) 1–3 within the family *Paramyxoviridae*, human metapneumovirus (HMPV), and human respiratory syncytial virus from the family *Pneumoviridae* were obtained and analyzed.

Results: M proteins were very diverse among HPIVs, but highly conserved within each virus. More variability was seen in nucleotide sequences of M proteins from the *Pneumoviridae* family. An insertion of 8 nucleotides in the 3' untranslated region in 1 HMPV M gene sequence was discovered (HR347-12). As there are no samples with such an insertion in the database, this insertion is of interest and requires further research.

Conclusion: While we have confirmed that M proteins were conserved among individual viruses, any changes that are observed should be given attention and further researched. Of special interest is inclusion of HPIV2 M proteins in this analysis, as these proteins have not been studied to the same extent as other paramyxoviruses.

Keywords: genetic variability, matrix protein, paramyxoviruses, pneumoviruses

9. GENOTYPE REPLACEMENT OF THE HUMAN PARAINFLUENZA VIRUS TYPE 2 IN CROATIA BETWEEN 2011 AND 2017 – THE ROLE OF NEUTRALISING ANTIBODIES

Epidemiology and Infection. 2018;146(11):1372-1383

Impact Factor: 2.044

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Abstract

Previously we reported on the HPIV2 genotype distribution in Croatia 2011–2014. Here we expand this period up to 2017 and confirm that G1a genotype has replaced G3 genotype from the period 2011–2014. Our hypothesis was that the G1a-to-G3 genotype replacement is an antibody-driven event. A cross-neutralisation with anti-HPIV2 sera specific for either G1a or G3 genotype revealed the presence of genotype-specific antigenic determinants. By the profound, *in silico* analyses three potential B cell epitopic regions were identified in the hemagglutinin neuraminidase (regions 314–361 and 474–490) and fusion protein (region 440–484). The region identified in the fusion protein does not show any unique site between the G1a and G3 isolates, five differentially

glycosylated sites in the G1a and G3 genotype isolates were identified in epitopic regions of hemagglutinin neuraminidase.

All positively selected codons were found to be located either in the region 314–316 or in the region 474–490 what indicates a strong positive selection in this region and reveals that these regions are susceptible to evolutionary pressure possibly caused by antibodies what gives a strong verification to our hypothesis that neutralising antibodies are a key determinant in the inherently complex adaptive evolution of HPIV2 in the region.

Keywords: genotype, fusion protein, hemagglutinin-neuraminidase, human parainfluenza virus type 2, neutralising antibodies

10. GEOCHEMISTRY OF CROATIAN SUPERHIGH-ORGANIC-SULPHUR RAŠA COAL, IMPORTED LOW-S COAL, AND BOTTOM ASH: THEIR SE AND TRACE METAL FINGERPRINTS IN SEAWATER, CLOVER, FOLIAGE, AND MUSHROOM SPECIMENS

International Journal of Oil Gas and Coal Technology. 2018;18(1-2):3-24

Impact Factor: 0.563

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Abstract

The Labin city area has represented the major Croatian coal mining, metal industry, and coal-fired electricity centre for more than two centuries. The domestic superhigh-organic-sulphur (SHOS) Raša coal is a unique variety compared to other coal types worldwide, based on its highest organic sulphur values, up to 11%. It was utilized in the Plomin coal-fired power plant during the period 1970-2000, and was replaced by an imported low-S coal afterwards.

This paper presents the levels of S, Se, V, U, Hg, Sr, Cd, Cr, Pb, Cu, and Zn in the two coal types, their bottom ash, seawater, and plant (clover, mushroom, and foliage) specimens collected from the Labin city area, while the sulphate was measured in surface stream water. Their levels were compared with relevant legislative as well as the published data from different world localities.

Data analysis was interpreted in the context of past and recent coal combustion activities.

Keywords: SHOS Raša coal, low-S coal, bottom ash, sulphur, selenium, uranium, vanadium, seawater, clover, foliage, mushroom, trace elements

11. MOLECULAR MECHANISMS OF *CHLAMYDIA TRACHOMATIS* RESISTANCE TO ANTIMICROBIAL DRUGS

Frontiers in Bioscience-Landmark. 2018;23:656-670

Impact Factor: 2.349

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Abstract

Chlamydia trachomatis (*C. trachomatis*) is a leading cause of bacterial sexually transmitted infections in developed and undeveloped countries, and therefore a global public health issue. In an era of increasing bacterial resistance to antibiotics, resistance has been an exceedingly rare phenomenon in *C. trachomatis*; however, clinical treatment failures attributed to multidrug-resistant *C. trachomatis* strains have been described on several occasions. Cell culture systems using McCoy cells and subsequent immunofluorescent staining are still the most common methodology used for antimicrobial susceptibility testing, but the presence of resistance markers should be appraised by further genetic analysis.

Azithromycin resistance of *C. trachomatis* is often a result of the mutations in the peptidyl transferase region of 23S rRNA genes, tetracycline resistance is usually linked to the presence of foreign genomic islands integrated in chlamydial chromosome, whereas a predominant mechanism of fluoroquinolone resistance is a point mutation in the *gyrA* quinolone-resistance-determining region.

A nucleotide substitution in *rpoB* gene is responsible for rifampin resistance, and different mechanisms have been involved in the development of resistance to aminoglycosides, lincomycin and sulphonamide/trimethoprim combinations.

Keywords: *Chlamydia trachomatis*, antibiotics, antimicrobial resistance, genetics, mutations, review

12. MORTALITY CHARACTERISTICS OF TWO POPULATIONS IN THE NORTHERN MEDITERRANEAN (CROATIA) IN THE PERIOD 1960–2012: AN ECOLOGICAL STUDY

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Abstract

In the second half of the 20th century, the town of Bakar (Primorje-Gorski Kotar County, Croatia), where a coking plant was operational 1978–1994, experienced intensive industrialisation. The town of Mali Lošinj (Primorje-Gorski Kotar County, Croatia) in this period based its economy on non-industrial sectors. The study goal was comparing mortality characteristics of these populations in the northern Mediterranean for 1960–2012. An ecological study design was used. Data were analysed for 1960–2012 for the deceased with recorded place of residence in the study area. Data on the deceased for 1960–1993 were taken from death reports, for 1994–2012 from digital archives of the Teaching Institute of Public Health, Primorje-Gorski Kotar County.

Data on causes of death for 1960–1994 were recoded to the three-digit code of underlying cause of death according to the International Classification of Diseases (ICD–10). Among studied populations significant difference was found

among the causes of deaths coded within ICD–10 chapters: neoplasms (particularly stomach carcinoma), mental and behavioural disorders and diseases of the respiratory system (particularly chronic obstructive pulmonary disease, (COPD)). Increase in mortality from neoplasms, increase in respiratory diseases for the area exposed to industrial pollution, also stomach carcinoma and COPD particularly in the town Bakar require further research.

Keywords: Mediterranean, Croatia, Bakar, Mali Lošinj, air pollution, mortality, diseases of the respiratory system, COPD, neoplasms, stomach carcinoma

13. PHARMACOLOGICAL AND TOXICOLOGICAL HEALTH RISK OF FOOD (HERBAL) SUPPLEMENTS ADULTERATED WITH ERECTILE DYSFUNCTION MEDICATIONS

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Abstract

Erectile dysfunction is one of the most common sexual disorders among man, with large number of patients worldwide. This is multifactorial disease with (non)organic causes while main oral drugs for the treatment are phosphodiesterase type 5 inhibitors (PDE5Is). Currently, sildenafil, vardenafil, tadalafil, avanafil, udenafil, mirodenafil, lodenafil and avanafil are used. Worldwide use of herbal and dietary supplements for alleviating this problem is on a rise, as many consumers perceive these products as “healthier” and safer as compared to conventional pharmaceutical preparations. However, adulteration of food supplements with PDE5Is and/or its analogues is very common and represents serious public health concern. This review address the issues with pharmacological and toxicological health risks of food supplements adulterated with erectile dysfunction medications.

Keywords: food supplements for erectile dysfunction; phosphodiesterase type 5 inhibitors (PDE5Is); adulterated sildenafil; tadalafil; vardenafil; avanafil

14. PHYSIOLOGICAL, ULTRASTRUCTURAL AND PROTEOMIC RESPONSES OF TOBACCO SEEDLINGS EXPOSED TO SILVER NANOPARTICLES AND SILVER NITRATE

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Abstract

Since silver nanoparticles (AgNPs) are a dominant nanomaterial in consumer products, there is growing concern about their impact on the environment. Although numerous studies on the effects of AgNPs on living organisms have been conducted, the interaction of AgNPs with plants has not been fully clarified. To reveal the plant mechanisms activated after exposure to AgNPs and to differentiate between effects specific to nanoparticles and ionic silver, we investigated the physiological, ultrastructural and proteomic changes in seedlings of tobacco (*Nicotiana tabacum*) exposed to commercial AgNPs and ionic silver (AgNO₃) from the seed stage.

A higher Ag content was measured in seedlings exposed to AgNPs than in those exposed to the same concentration of AgNO₃. However, the results on oxidative stress parameters obtained revealed that, in general, higher toxicity was recorded in AgNO₃-treated seedlings than in those exposed to nanosilver. Ultrastructural analysis of root cells confirmed the presence of silver in the form of nanoparticles, which may explain the lower toxicity of AgNPs. However, the ultrastructural changes of chloroplasts as well as proteomic study showed that both AgNPs and AgNO₃ can affect photosynthesis. Moreover, the majority of the proteins involved in the primary metabolism were up-regulated after both types of treatments, indicating that enhanced energy production, which can be used to reinforce defensive mechanisms, enables plants to cope with silver-induced toxicity.

Keywords: silver nanoparticles, *Nicotiana tabacum*, phytotoxicity, ultrastructure, proteomics

15. PHYTOTOXIC EFFECTS OF SILVER NANOPARTICLES IN TOBACCO PLANTS

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Abstract

The small size of nanoparticles (NPs), with dimensions between 1 and 100 nm, results in unique chemical and physical characteristics, which is why they are implemented in various consumer products. Therefore, an important concern is the potential detrimental impact of NPs on the environment. As plants are a vital part of ecosystem, investigation of the phytotoxic effects of NPs is particularly interesting. This study investigated the potential phytotoxicity of silver nanoparticles (AgNPs) on tobacco (*Nicotiana tabacum*) plants and compared it with the effects of the same AgNO₃ concentrations. Accumulation of silver in roots and leaves was equally efficient after both AgNP and AgNO₃ treatment, with predominant Ag levels found in the roots. Exposure to AgNPs did not result in

elevated values of oxidative stress parameters either in roots or in leaves, while AgNO₃ induced oxidative stress in both plant tissues. In the presence of both AgNPs and AgNO₃, root meristem cells became highly vacuolated, which indicates that vacuoles might be the primary storage target for accumulated Ag. Direct AgNP uptake by root cells was confirmed. Leaf ultrastructural studies revealed changes mainly in the size of chloroplasts of AgNP-treated and AgNO₃-treated plants. All of these findings indicate that nano form of silver is less toxic to tobacco plants than silver ions.

Keywords: silver nanoparticles, *Nicotiana tabacum*, oxidative stress, comet assay antioxidant enzymes, ultrastructure

16. SEROEPIDEMIOLOGICAL STUDY OF EPSTEIN-BARR VIRUS IN DIFFERENT POPULATION GROUPS IN CROATIA

Israel Medical Association Journal. 2018;20(2):86-90

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Abstract

Background: The Epstein-Barr virus (EBV) is one of the most common viruses found in humans, causing lifelong infection in up to 95% of the world population.

Objectives: To analyze the seroprevalence of EBV infection in different population groups in Croatia.

Methods: During a 2-year period (2015-2016), a total of 2022 consecutive serum samples collected from Croatian residents were tested for the presence of EBV-specific viral capsid antigen (VCA) immunoglobulin M (IgM) and IgG antibodies using an enzyme-linked immunoassay. IgM/IgG-positive samples were further tested for IgG avidity.

Results: The overall prevalence of EBV IgG antibodies was 91.4%. Females had significantly higher IgG seroprevalence than males (93.1% vs. 89.9%, $P = 0.008$). According to age, IgG seropositivity increased progressively from 59.6% in children age < 9 years to 98.3% in 30-39 year olds, and remained stable thereafter

($P < 0.001$). The IgG seroprevalence differed significantly among groups: 68.1% in children/adolescents and 95.9% in adults; multiple sclerosis (100%), haemodialysis patients (97.7%), heart transplant recipients (93.8%), haematological malignancies (91.2%), and Crohn's disease (88.5%), $P < 0.001$. IgM antibodies were detected in 9% of participants. Using IgG avidity, recent primary EBV infection was documented in 83.8% of IgM-positive subjects < 9 years old, 69.2% age 10-19, 33.3% age 20-29, and 3.6-4.2% > 40 . All IgM positive participants > 40 years showed high IgG avidity. Logistic regression showed that age is associated with EBV IgG seropositivity.

Conclusions: EBV is widespread in the Croatian population. Older age appears to be the main risk factor for EBV seropositivity.

Keywords: Epstein-Barr virus (EBV) seroprevalence, Crohn's disease, multiple sclerosis, transplantation, haemodialysis

17. THE EFFECT OF HAZARDOUS POLLUTANTS FROM COAL COMBUSTION ACTIVITY: PHYTOTOXICITY ASSESSMENT OF AQUEOUS SOIL EXTRACTS

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Abstract

Airborne fly ash and related hazardous particles derived from coal combustion contaminate soil and groundwater, negatively affecting ecosystems. The aim of this study was chemical and toxicological evaluation of aqueous extracts of soil collected from the vicinity of a coal-fired Plomin power plant (PPP), using *Lemna* (*Lemna minor* L.) bioassay and additional biochemical indicators – photosynthetic pigments, lipid peroxidation, antioxidative enzymes and glutathione. Topsoil samples were collected from distances of 200, 300, 400 and 800 m from the PPP in accordance with the prevailing SW wind direction. Elevated levels of polycyclic aromatic hydrocarbons (up to 15, 765 ng L⁻¹) and potentially toxic trace elements were detected in the Plomin soil extracts (PEs) in comparison to control soil extract (CE). Trace elements accumulated in *L. minor* were mostly in accordance with their concentrations in PEs. The results demonstrate that PEs induced significant growth inhibition and other phytotoxic effects. Those effects can be related to damage caused by increased production of reactive oxygen species and impaired antioxidant levels.

The connection among the phytotoxicity, a distribution of analysed contaminants, and distances from the PPP is clearly established.

Keywords: toxicity, duckweed, metal, organic pollutant, coal

18. VARIABILITY ANALYSIS AND INTER-GENOTYPE COMPARISON OF HUMAN RESPIRATORY SYNCYTIAL VIRUS SMALL HYDROPHOBIC GENE

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Abstract

Background: Small hydrophobic (SH) gene is one of the mostly diverse genomic regions of human respiratory syncytial virus (HRSV). Its coding region constitutes less than 50% of the complete gene length, enabling SH gene to be highly variable and the SH protein highly conserved. In standard HRSV molecular epidemiology studies, solely sequences of the second hypervariable region of the glycoprotein gene (HVR2) are analyzed. To what extent do the strains identical in HVR2 differ elsewhere in genomes is rarely investigated. Our goal was to investigate whether diversity and inter-genotype differences observed for HVR2 are also present in the SH gene.

Methods: We sequenced 198 clinical samples collected within a limited area and time frame. In this HRSV collection, rapid and significant changes in HVR2 occurred.

Results: Over 20% of strains from this pool (containing HRSV genotypes NA1, ON1, GA5, BA9 and BA10) would be incorrectly assumed to be identical to another strain if only the HVR2 region was analysed. The majority of differences found in SH gene were located in the 5' untranslated region (UTR). Seven indels were detected, one was genotype GA5 specific. An in-frame deletion of 9 nucleotides (coding for amino acids 49-51) was observed in one of group A strains. Fifteen different SH protein sequences were detected; 68% of strains possessed the consensus sequence and most of others differed from the consensus in only one amino acid (only 4 strains differed in 2 amino acids). The majority of differing amino acids in group A viruses had the same identity as the corresponding amino acids in group B strains. When analysis was restricted to strains with identical HVR2 nucleotide sequences and differing SH protein sequences, 75% of differences observed in the SH ectodomain were located within region coding for amino acids 49-51.

Conclusions: Basing HRSV molecular epidemiology studies solely on HVR2 largely underestimates the complexity of circulating virus populations. In strain identification, broadening of the genomic target sequence to SH gene would provide a more comprehensive insight into viral pool versatility and its evolutionary processes.

Keywords: human respiratory syncytial virus, HVR2, molecular epidemiology, molecular evolution, SH, small hydrophobic gene, virus variability

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