

EFSA SCIENTIFIC COOPERATION AND NETWORKING ACTIVITIES

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ABSTRACT

EFSA's Strategy 2020 highlights "Cooperation" as one of the five fundamental key values under which EFSA currently guides its activities. This has been so in the past and is progressively evolving in nature, with Member States playing more and more a leading role on priority setting and steering of activities.

This presentation briefly describes the most relevant ongoing and upcoming scientific cooperation activities undertaken by EFSA in collaboration with different stakeholders, in particular with Member States. It also highlights how EFSA supports the European Commission, its main stakeholder, in policy-making decisions and enlargement programmes, contributing to the harmonisation of risk assessment practices at a European and international scale.

The activities of the Advisory Forum and of its Working Groups, as a platform for providing strategic advice to the Executive Director, the visits to Member States of EFSA's Executive Director and the respective outcomes, the implementation by EFSA of innovative grant and procurement activities, including the current Focal Point Agreements and the management of the Article 36 List, are all topics that will be touched upon. Other issues to be addressed are the ongoing and upcoming plans for training in risk assessment, the activities of EFSA's Scientific Networks, the Instrument for Pre-Accessions countries and the European Neighbourhood Programme, and finally EFSA's International Scientific cooperation activities.

Scientific cooperation must be nurtured and actively pursued to provide useful results and catalyse real change. Sharing information and expertise is the path to impact and to innovation, but we must all engage in this process, as it is the result of a group effort and no single player has the power to make it happen. A key development in our work today is that EFSA is facilitating rather than leading cooperation. We look to our partners, our stakeholders, our international peers for guidance and support, to spark debate and innovation.

Fostering EU and international cooperation will strengthen EFSA's role to promote the highest standards of excellence in risk assessment.

RISK ASSESSMENT AND FOOD SAFETY

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ABSTRACT

International agencies define food safety as all actions aimed at ensuring that all food is as safe as possible. Food safety policies and actions need to cover the entire food chain, from production to consumption (http://www.who.int/topics/food_safety/en/). Ensuring food safety is a task that every government should undertake using a science-based approach. This includes policies and activities to ensure food safety in the food chain, from production to consumption. Every year, millions of people are exposed to physical, chemical or biological hazards deriving from food. These hazards may be due to factors such as abuse and uncontrolled use of agrochemicals, environmental pollutants, use of unauthorized substances, and inappropriate control practices. The increasing globalization of food trade, urbanization, changing consumption patterns, the intensification of agriculture, and new types of production and manufacturing systems are just some of the trends that are having a serious impact on food safety in many countries.

The study of the relationship between the presence of a hazard in a food and a health risk is called risk assessment. Risk assessment allows the improvement of food standards and management of food safety through a comprehensive scientific assessment, involving several stakeholders, and using a transparent process. A modern food safety system includes a risk analysis approach that allows a fast response to emergencies and also manages focussed interventions to properly deal with rising food safety challenges.

Risk analysis has been defined in the Codex Procedural Manual as a process consisting of three components: risk assessment, risk management and risk communication (CAC, 2003. Procedural Manual, 13th Edition). Each of these components plays an essential and complementary role in the risk analysis process. Codex defines risk assessment as a scientifically based process consisting of four steps: i) hazard identification; ii) hazard characterization; iii) exposure assessment; and iv) risk characterization. There is more than one way to perform a food safety risk assessment.

Different models for food safety risk assessment exist and the process will vary according to the type of risk, the model used and the questions to be answered. For residues of pesticides and veterinary drugs in food, the risk assessors determine an acceptable daily intake (ADI). On the basis of the ADI and Good Agricultural Practices, risk managers can set maximum residue levels (MRL) for pesticides and veterinary drugs.

To be effective a science-based risk analysis framework should cover food safety legislation, food inspection, laboratory analysis, monitoring systems for chemical and microbiological contamination of foods, and information, education and communication (<http://www.fao.org/docrep/006/y8705e/y8705e04.htm>). In this food control system the role

of the laboratory is essential to ensure quality data that can be made available for risk analysis purposes.

In the field of analytical techniques and food control mechanisms, the Joint FAO/IAEA Division supports countries in using nuclear-related and complementary technologies to detect and monitor chemical residues and contaminants in food and the environment, thereby assisting in providing data for risk assessment and provision of technical support to risk assessment bodies.

Nuclear techniques can be used to advantage in a number of ways in food safety and environmental protection applications. Radiolabelled compounds can be used as radio-tracers to optimize sample preparation, extraction, clean-up and other steps during the development of analytical methods that are used in regulatory programmes for the analysis of residues of pesticides and other contaminants in food and environmental samples. Radiolabelled compounds can assist in improving method performance characteristics and the estimation of uncertainty associated with the method. Stable isotope-labelled compounds can be used as internal standards to improve the precision and accuracy of analytical methods using mass spectrometry, to meet stringent international or national standards and trading requirements. The real power of nuclear and related techniques is in their application in a package along with complementary, non-nuclear techniques, to provide solutions to complex analytical problems and reliable data to inform risk assessment bodies for decision making in a policy or regulatory context.

In summary, the use of a science-based risk analysis approach will enable governments to develop and implement a range of general improvements and interventions tailored to specific risk areas, which will ultimately improve food safety and increase food trade. A pesticide residue risk assessment process will be discussed to demonstrate the importance of a preventative approach to ensure food safety and environmental sustainability.

THE REPUBLIC OF MACEDONIA ON ITS WAY TO THE EU BUILDING UP OF THE FOOD SAFETY SYSTEM IN COOPERATION WITH EFSA

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ABSTRACT

The food safety is a significant public health issue for the governments around the world. Effective national food control systems are essential to protect the health and safety of consumers. In this direction, the risk assessment approach provides an objective scientific basis for decision making in ensuring the safety of the food chain. However, the process of establishment of risk assessment procedures and methodologies might present a challenge that developing countries need to overcome. In February 2008 the Republic of Macedonia started to cooperate with the European Food Safety Authority (EFSA) within the framework of EFSA Pre-Accession Programme entitled: "Preparatory Measures for the Participation of the

Candidate Countries and the Potential Candidate Countries in EFSA. The overall objective of the Programme is to promote understanding of EFSA's work in the Pre Accession Countries, share expertise, create information exchange mechanisms, and involve the national authorities in crisis coordination exercises. Competent Authorities representatives, scientific and communication experts participated in a number of events organised by the Pre-Accession programme like trainings on risk assessment in different food safety areas, workshops/seminars and study tours to the EU Member States (MSs). During the period from 2011-2014 the total number of organized events for the Republic of Macedonia was 68 with participation of 125 Macedonian food safety experts. Study tours were organised to follow up on the country need to get an overview on national food safety systems from the EU MSs. In particular, these events provided a comprehensive overview of the legal, regulatory and administrative aspects of national food safety systems in the EU MSs. In the recent years, the food safety issues in the Republic of Macedonia have received an increased attention from both the food safety authorities and the consumers. With the aim of establishing an effective national food control system, which is not only necessary to protect consumers, but to also ensure the safety and quality of exported and imported food, the restructuring of the food safety authorities took place. The Food and Veterinary Agency of the Republic of Macedonia (FVA) was established on 1st of January 2011. This institution is the legal successor of former two institutions, the Food Directorate under the Ministry of Health and the Veterinary Directorate under the Ministry of Agriculture, Forestry and Water Economy. FVA is an independent Governmental Authority that coordinates all food and feed safety related activities within the Republic of Macedonia and is also responsible for the communication with the EU institutions. FVA is responsible for centralized data collection of risk/hazards related to the food chain in accordance with provisions of the Food Safety Law. The result of the national endeavors and international assistance has largely contributed to the improvement of the food and feed safety in the Republic of Macedonia. This has been continuously noted in the European Commission annual progress reports where, for the year 2015, in the area of food safety, veterinary and phytosanitary policy some level of preparation has been achieved. In addition, some progress was made, particularly related to food safety issues. This progress report also helps the Republic of Macedonia, as a Candidate Country for accession to the European Union, to fulfill the requirements of the EU food safety legislation and to better prepare for the very accession. In the next period, the country should increase the administrative capacities, in particular of inspection services and laboratories, as well as the risk assessment capacity. In addition, in order to reach the EU standards, the capacities for data collection and exchange should be improved. Finally, more active involvement of the scientific community in decision making process should be encouraged. The successful cooperation with EFSA and continuation of the Pre-Accession Programme for capacity building will certainly provide an additional opportunity for improvement of the national food safety system and protection of consumers in general.

THE STATE OF GENETIC RESOURCES AND CONSERVATION IMPLICATIONS OF ENDEMIC AND EXTREMELY ENDANGERED BULGARIAN PLANT SPECIES FROM VERBASCUM GENUS WITH PHARMACOLOGICAL POTENTIAL

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A significant part of available bioresources in our planet consists of medicinal plants with ethnobotanical and economical value. Biodiversity provides important resources for traditional and modern medicine. Unfortunately, its loss may have significant and direct impact on human health. Plant diversity on the territory of Bulgaria is remarkable. Almost 12% of Bulgarian plant species are endemic to Bulgarian or Balkan flora, and approximately 19% are of pharmaceutical value. Today many medicinal plants face extinction, due to severe genetic loss. The strategic goal of our study is to assess the nowadays genetic resources of three endangered plant species with pharmacological potential - *Verbascum tzar-borisii*, *Verbascum davidoffii* and *Verbascum anisophyllum*, all belonging to the *Verbascum* genus, one of the richest in endemic plants in Bulgarian flora. The anthropogenic disturbance on species' habitats, the low reproductive potential of these plants, as well as the restricted distribution and the small size of their populations are the major drivers, which led to the risk of extinction. In order to conserve these critically endangered plants, the study on the level and distribution of their genetic diversity patterns is becoming necessary and timely. Molecular analyses were applied to elucidate genetic diversity and relationship within and among the existing nowadays populations of these *Verbascum* species in Bulgaria. Our pool of molecular data provide a very important information on genetic basis. Beyond the stability of ecosystems and in situ preservation, special measures for conservation of these endangered plants and sustainable utilization of their available genetic resources are necessary.

ALGAE AND FUNGI AS NOVEL FOODS AND FOOD SUPPLEMENTS

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ABSTRACT

Due to the globalization, food habits and dietary patterns of many people in Bulgaria have been radically changed. Recently in the country new food and food supplements have become readily available on the actual food market and their consumption has increased significantly. Among them appeared many different species of algae and fungi with important

role in the human life. They are imported in Bulgaria mainly from Asia but now some of them are successfully cultivated in the country and are used as a new healthy food of the modern man. The algal and fungal food and food supplements offer different and exotic taste and in the same time they have powerful medicine effect. Therefore the presentation provides data for the using of algal and fungal products in Bulgaria.

API-TEST OF LACTOBACILLI ISOLATED FROM TOP BRANDS COMMERCIAL YOGURT

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ABSTRACT

Lactic acid bacteria are pronounced probiotics. They strongly improve the human immune system and produce beneficial for the human health vitamins. Lactobacilli shows strong bactericidal and antifungal activity. Due to synthesis of many enzymes involved in degradation of toxic substances yogurt is known as a food of long-livers. Lactobacilli of six top brands of yogurt, available at the bulgarian market - "Vereya", "Na baba", "Elena", "LB", "Parshevitza" and "Rodopeya" were examined using APItest for their biochemical activity. Lactobacilli were isolated on MRS media initially enriched with skim milk. Analysis was conducted via API 50 CH kit (Biomerieux, France) testing degradation of 50 different metabolites: glycerol, erythrol, D-and L-arabinose, L-arabinose, D-ribose, D- and L-xylose, D-adonitol, methyl-beta-D-xylopiranoside, D-galactose, D-glucose, D-fructose, D-mannose, D-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-alpha D-mannopyranoside, methyl-alpha D-glucopyranoside, N-acetilglucosamin, amygdalin, arbutin, esculin/ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inulin, D-melizitose, D-rafinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, potassium guconate, potassium 2-ketogluconate, potassium 5-ketogluconate ribose. Our results demonstrated that isolated lactobacilli degrade 4 metabolites - D-glucose, D-fructose, D-mannose and D-lactose and belong to *Lactobacillus bulgaricus* ssp. *delbrueckii*.

Key words: Lactobacilli, API-test, commercial yogurt

RISKS DURING "CRAFT" BEER PRODUCTION

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ABSTRACT

"Craft" beers represent the production of homemade beer from amateurs and from micro and small breweries. Often these beers in Bulgaria are called „live“, this term and their use will be discuss in our presentation. According to Bulgarian legislation, the beer

production from breweries with capacity under 10 000 hl., belong to these categories of beer, as well as the homemade beer produced for own consumption. During the past years, homemade beer production became more popular. Many of small producers offer for sale their beers through shops or enter-net. Commonly they advertise their beers as alternative of “industrial”. Nevertheless, this tendency might be dangerous for craft beer consumers. In this presentation the risks during the homemade beer will be describe. The results from microbiology and chemical investigations of craft beers will be presented and discuss and same recommendations for safety increase will be given.

Key words: “Craft” beer, “live” beer, microbrewery

ASSESSMENT OF BYOLOGICALY ACTIVE AND TRANS FATTY ACIDS IN FAT FRACTION ON THE COW’S YOGURT

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ABSTRACT

Natural sources of trans isomers of fatty acids are primarily the milk fat and other fats from animal origin. The study was conducted with sour milk containing 2, 3 and 4.5% for the determination of biologically active and trans fatty acids and qualitative assessment of the fat fraction. Yogurt with 4,5% fat have a highest content of SFA- 3,13 g / 100g product, MUFA- 1,18 g / 100g product, PUFA-0,15 g / 100g product, oleic- 0,98 g / 100g product, linoleic-0,08 g / 100g product, trans fatty acids- 0,14 g / 100g product, CLA-0,03 g / 100g product as long as 2% yogurt is the poor of biologically active fatty acids- oleic acid- 0,43 g / 100g product, linoleic- 0,04 g / 100g product, trans fatty acids- 0,09 g / 100g product, CLA-0,01 g / 100g product. Lipid preventive score, index of atherogenicity and thrombogenicity is highest at 4,5% yogurt - 9,50 g / 100g product, 3,02 and 2,82. The analyzed yoghurts are characterized as a food product with a low content of trans fatty acids - 0,06 to 0,14 g / 100g product and a low content of saturated fatty acids in the yogurt with 2% fat-1,45.

Key word: yogurt, trans fatty acids, CLA

THE ROLE OF MICROBIAL FACTOR FOR PRODUCTION OF HISTAMINE IN FOOD

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ABSTRACT

The histamine occupies a leading position among the biogenic amines in foodstuffs. The high stability of these amines in food processing technology represents a real health risk

to the consumer. The expert studies on histamine are primarily motivated by registered numerous cases of the food poisoning after consuming of risk foods in certain regions of the world. The intensive international trade of fish, meat and dairy products enables the distribution of dangerous foods, containing high levels of histamine throughout the food chain. The current database for risk assessment and content of histamine in food is insufficient. The information on the toxic effect, dose response and the actual content of histamine in the main groups of risk foods, available on the market, are limited. The chemical structure of histamine and its biological action on the human are subject of the research through the years a variety of experimental research laboratories. It was found that the biogenic amines, including histamine, are involved in normal physiological processes in the human body and animals. The histamin acts as neurotransmitter that is actively involved in inflammation and allergic reactions of the body and is stored in immunocompetent cells /mast cells/. The same is also relevant to cell growth. Practically the main receipt of histamine is achieved through food products / fish, meat, dairy and others./ containing various concentrations. Most often these are foods undergone a process of microbial fermentation. Along with histamine in these foods are found other biogenic amines formed during the microbial metabolism - tyramine, cadaverine, putrescine, phenylethylamine and others. The role of microbial factor in the production of toxic metabolites in foods is proven unconditionally. It is known that a number of microorganisms are forming enzymes which are decarboxylated the amino acids to amines. The amount and type of these compounds is affected by category and the composition of the food. The importance is placed on the temperature and duration of storage of food, qualitative and quantitative composition of the microflora, the possibility of bacterial growth, the pH, water activity and others. The conducted scientific studies on the ability of certain groups of microorganisms to decarboxylate the histidine testify that among of them are found both Gram-positive and Gram-negative bacteria. High values of histamine have been identified in the degradation of amino acids from various representatives of the genus Klebsiella, Hafnia, Morganella, Pediococcus, Lactobacillus, Leuconostoc, Photobacterium, Pseudomonas, Enterococcus, Staphylococcus, Bacillus etc. The majority of these microorganisms have psychrophilic and psychrotrophic nature of growth. The continued storage of food at improper temperatures in the different stages of the food chain creates suitable conditions for the development of the microflora and the possibility of formation of significant quantities of histamine. Particularly risky in this respect are poorly refrigerated fish raw materials whose supply chain is complicated and difficult to control. It is recommended proper cooling of the fish with ice and cold air to suppress the synthesis of bacterial proteases and decarboxylases during transport and storage of the product as a preventive measure. The activity of the decarboxylase is affected by the dynamics of the pH in food. These are established two types of influence of pH, which act simultaneously. In one type, the reduction in acidity leads to active inhibiting the growth of microorganisms. In the other type is found increased production of the enzymes in an acidic environment, including decarboxylase, as a defense mechanism of microorganisms from adverse environmental. These mechanisms influence each other and the end result is determined by their balance. Different impact on histidine decarboxylase activity of different types of bacteria has the presence of salts in the product. The high content of salt in certain fishery products inhibit the growth of non-halophilic microflora. At the same time

the halophilic staphylococci and bacillus activated a production of their decarboxylase. The clarification of the microbial metabolism, associated with histamine, requires the development of appropriate models and techniques for detection of microorganisms which forming a histamine. The experiments, using different culture media, were found to be unsuccessful due to the high percentage of false positive and false negative results. The phenotypic characteristics of potential histamine-producers is slow and uncertain approach. Therefore more and more specialists rely on a molecular-biological methods in the study of a specific genes, encoding a bacterial decarboxylase and the ability to synthesize specific biogenic amines. The detection of these genes by PCR-based methods will allow for easier demonstration of the microorganisms producers of histamine and others biogenic amines. Among the positive aspects of this modern technique, as a disadvantage of the PCR method it is stated that it can be identified a certain potential for the formation some amines, but not an a real production of biologically active enzymes. In order to overcome the shortcomings of various microbiological approaches are considered appropriate first to isolate the microorganism, and then proceed to detect specific genes associated with the production of histamine. Special importance is placed on PCR-technique for typing and selecting strains of microorganisms included as members of starter cultures in the production of a variety of assortments of fish, meat and dairy products with a fixed ripening period. The hygienic and health importance of histamine is assessed by the fact that the microbiological criteria for foodstuffs laid down in Regulation (EC) №2073/2005 have introduced specific safety regulations. For now, these criteria comprises only for fish from six families /Scombridae, Clupeidae, Engraulidae, Coryphenidae, Pomatomidae и Scombrosidae/, with a high content of the amino acid histadine, as well as a fish of these families, which have undergone enzyme treatment in brine. The studies have shown that these foods have a high priority for assessment of their health safety, associated with the presence of specific quantities of histamine. The studies on the role of microbial factor in the production of histamine in different foods give reason to accept that there is a need to optimize the microbiological criteria and expansion of their scope to other hazardous food /meat and dairy products/, occupying a considerable share of the food industry in the EU.

POTENTIAL RISKS OF USING FOOD SUPPLEMENTS WITH ALGAE

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ABSTRACT

Algae are a valuable source of nutrients and bioactive compounds. For more than 50 years, algae-containing supplements with various forms and names have been available

among the assortment of specialized stores around the world. Generally, food supplements are substances of natural or artificial origin which are not used as food or as a predominant ingredient in food production. They can complement the normal diet and they are also concentrated sources of beneficial substances. Food supplements, alone or in combination are dosed in the form of capsules, tablets, powders, ampoules, syrups, etc. and they should be taken in small amounts. It has been proven that the biomass of certain algal species such as *Spirulina* sp., *Chlorella* sp., *Scenedesmus* sp., *Dunaliella salina*, *Haematococcus pluvialis*, etc. has a positive effect on human health as a result of its well-balanced biochemical composition - high content of proteins, polysaccharides, essential fatty acids, vitamins, pigments, sterols, and a number of other biologically active substances (Gouveia et al., 2008). *Spirulina* has been used as food for thousands of years and *Chlorella* - for more than 80 years. Food supplements containing these algal species are highly preferred - the production of *Spirulina* and *Chlorella* is 5000 t and 2000 t per year, respectively. On the other hand, valuable metabolites derived from algal biomass having numerous physiological effects (astaxanthin, β -carotene, omega-3 fatty acid, etc.) are produced in a much more limited scale; however, they have a significant marketing potential (Enzing et al. 2014). Therefore, it can be concluded that algae is a sustainable raw material for the preparation of food supplements. In addition to the benefits, the use of algae-containing food supplements might be dangerous for human health. The risks may arise both from the nature of algae themselves and from some mistakes in manufacturing. Under certain conditions some algae, e. g. *Aphanizomenon flos-aquae* (AFA), can produce toxins so their usage as food supplements could cause serious problems. For this and other reasons, the dietary supplements containing algae harvested from the wild should be avoided. There are also some risks when algae are produced in bioreactors. For example, cultivation in undefined nutrient media can lead to accumulation of heavy metals, pathogen microorganisms and toxins during the development of some adverse algal species. The use of heavy metals' salts, antibiotics and other biocides to control contaminants during the algal manufacturing process certainly leads to their accumulation in the biomass. The increase in moisture in the final product and improper storage of biomass can be also dangerous. Unfortunately, in our country and globally, there are no reliable standards for the quality of algal biomass and food additives produced on its basis. The aim of this report is to summarize and to discuss some of the common risks associated with the production of algal supplements, and also to propose quality parameters that ensure their safe usage of.

Key words: *food supplements, algae, quality parameters, risks, human health*

CHANGES IN THE EWE'S MILK COMPOSITION AND CONTENT OF BIOLOGICAL ACTIVE SUBSTANCES IN THE FAT FRACTION DURING THE GRAZING PERIOD

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ABSTRACT

The presented study is addressed to the growing market interest to the ewe's milk as a natural source of essential components, like conjugated linoleic acids (CLA) ω -3 and ω -6 polyunsaturated fatty and vaccenic acid, described to be effective substances for prevention of different human diseases. The work was focused on the transfer of certain organic components (fatty acids) in the food chain "plants–animal products" in the endemic regions of the Rhodope Mountain. The investigation emphasizes on the role of nutrition on the variation in the fatty acid profile during the grazing period. The feeding regime of the Rhodope Tsigai breed with essential nutrients and substrates through pasture vegetation is subjected to large variation depending on geochemical conditions, botanical diversity, season and stage of plant maturity. The submitted multidisciplinary investigation offers new knowledge in the field of healthy nutrition of sheep raised in mountain regions and the dynamic changes of organic nutrients (saturated and unsaturated fatty acids) in the meadow vegetation during the grazing period. With the detailed mapping of the pasture areas, an evaluation of exactly distribution of unsaturated and saturated fatty acids in the meadow vegetation as important substrate for the following transformation in the animal organism in form of biological active, isomers - conjugated fatty acids (CLA), vaccenic acid, ω -3 and ω -6 polyunsaturated fatty acids can be provided. The detailed study of essential fatty acids, especially its derivatives - conjugated linoleic acids (CLA) and natural trans fatty acids in the ewe's milk took an important place in the investigation. The dynamic changes in the available precursors in plant species affect the MUFA and PUFA-profiles of ewe's milk and provide additional information to clarify the mechanism of CLA-synthesis and the content of natural trans fatty acids in raw milk.

Key words: *ewe's milk, fatty acids, anticarcinogenic substances (CLA), trans-, ω -3 and ω -6 FA*

THE BACTERIUM XYLELLA FASTIDIOSA: A NEW PHYTOSANITARY RISK FOR BULGARIA

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ABSTRACT

Xylella fastidiosa (Wells et al., 1987) is a xylem-limited bacterium that is exclusively transmitted by sap-feeding hemipteran insects. Strains of this bacterium are endemic in the Americas and cause economically important diseases on different plants: Pierce's disease in grapevine, leaf scorch on almond, elm, oak, maple, pear, oleander, sycamore, and mulberry, phony peach disease and citrus variegated chlorosis. *X. fastidiosa* is distributed in the Americas, Asia (India, Iran, Lebanon, Taiwan), Europe (Southern Italy, Corsica, Southern France). The pathogen has four known subspecies: *X. fastidiosa* - *fastidiosa*, *pauca*, *multiplex* and *sandyi*, three of them have been reported in Europe. The different subspecies of *X. fastidiosa* affect various hosts in North and South America. In October 2013, the occurrence of *X. fastidiosa* subsp. *pauca* was reported for the first time in the EU, in southern Italy (province of Lecce, Apulia region) and was associated with quick decline symptoms on olive trees (Complesso del Disseccamento Rapido dell'Olivio) CoDiRO). At the end of 2013 the disease was found to affect about 23,000 hectares of olive groves in Apulia. *X. fastidiosa* was also found on *Prunus dulcis*, *Nerium oleander*, *Myrtus communis*, *Acacia saligna*, *Prunus avium*, *Polygala myrtifolia*, *Vinca* sp., etc. As a result of the implementation of Decisions of the European Commission from 2014 to 2015, emergency measures were conducted in Italy which included: Destruction of 2530 host plants located near roads, canals, green areas; monitoring of vectors on wild herbs and weeds; phytosanitary treatments for control of adult vectors; removal of infected olive plants in an area of 10 800 hectares; visual inspection, testing and destruction of host species in nurseries. In 2015 *X. fastidiosa* subsp. *multiplex* was identified in Corsica and Southern France on a large number of host species: *Polygala myrtifolia*, *Acer pseudoplatanus*, *Hebe* sp., *Lavandula* spp., *Myrtus communis*, *Pelargonium graveolens*, *Prunus cerasifera*, *Quercus suber*, *Rosa floribunda*. In 2016 *X. fastidiosa* subsp. *fastidiosa* was detected on a single potted plant of *Nerium oleander* in a small nursery stock producing glasshouse in Saxony, Germany. Additionally, *Coffea* plants originating from Central and South America contaminated by *X. fastidiosa* were intercepted by German, Dutch, French, Italian and Switzerland's official services (EPPO 2012). *Coffea* spp. and *N. oleander* plants are found to be susceptible to several subspecies (*fastidiosa*, *pauca*, *sandyi*) of *Xylella fastidiosa*. Since 2002, surveys of *X. fastidiosa* on vine (nurseries and vineyards) and vine plantations with imported and local propagation material have been conducted in Bulgaria. In compliance with Decision 2014/87/EU (and 2014 497/EU) of the European Commission (EC) on emergency measures to prevent the introduction into and the spread within the EU of *X. fastidiosa*, since July 2014 the Member States must carry out regular surveys within the Union territory on a wide range of host plants. An updated list of susceptible host plants of *X. fastidiosa* on the territory of the Union is available on the website of the Commission. In Bulgaria *X. fastidiosa*, is subject to regular surveys in orchards,

vineyards, ornamental and forest nurseries, greenhouses, botanical gardens, garden centers, parks and urban green spaces, and retail shops for plants for planting. The surveys also cover host plants originating from third countries and Member States, with particular emphasis on those originating from Italy and France. During the period July, 2014 – June, 2016, 2268 visual examinations and 505 laboratory tests of host plants of *X. fastidiosa* were conducted on the territory of Bulgaria. The largest shares of visual examinations (20.5%) and laboratory testing (66.5%) were performed for vines (propagation material and vineyards), followed by fruit trees (nurseries and orchards). So far *X. fastidiosa* has not been established on the territory of the country and in imported plant material. *X. fastidiosa* is transmitted from plant to plant by xylem-fluid feeding hoppers (Hemiptera). Even though different strains are transmitted by different species of hoppers, no specific relationships between the pathogen and the vector have been established. The transmission of *X. fastidiosa* by insects is peculiar and does not require a latent period. No transstadial or transovarial transmission has been observed. The bacteria are persistent in adults and multiply in the foregut of vectors. Nymphs and adults vectors acquire the bacteria by feeding in the xylem of infected plants and can inoculate the pathogen in healthy plants immediately after acquisition (Almeida et al., 2005). The transmission and spread of *X. fastidiosa* are mostly dependent on winged adults, because of their high mobility. As the bacterium is restricted to the foregut of the vector, the amount of bacterial cells per insect is very low. Therefore specific and sensitive diagnostic tools such as molecular methods are needed to detect the presence of *X. fastidiosa* in insect vectors. Until now, the hemipteran *Philaenus spumarius* has been identified as the only confirmed vector in Apulia, Italy (Saponari et al., 2014). Species in the families Cicadellidae, Aphrophoridae and Cercopidae should be regarded as potential vectors of the pathogen in Europe, as they are vectors of *X. fastidiosa* in the Americas. The hoppers from families Cicadidae and Tibicinidae should also be considered as potential vectors. For categorization of the potential vectors EFSA (2015) used the following three criteria: polyphagy, abundance and frequency in different environments. The list of hemipteran species distributed in Bulgaria and potential vectors of *X. fastidiosa* includes 27 species from 6 families: Cicadellidae, Aphrophoridae, Cercopidae, Cicadidae, Tibicinidae and Membracidae. Based on EFSA's criteria, the potential vectors of *X. fastidiosa* in our country are divided in three categories: very likely, likely and unlikely. The potential role of *Cicadella viridis* (Cicadellidae); *Aphrophora alni*, *Lepyronia coleoptrata*, *Neophilaenus campestris*, *Philaenus spumarius* (Aphrophoridae); *Stictocephala bisonia* (Membracidae) as vectors is considered very likely. Like in Italy, *P. spumarius* has the highest potential as a vector of *X. fastidiosa* in Bulgaria, due to its wide host range, distribution covering the whole country and its ability to develop extremely large populations in diverse agro- and ecosystems.

Key words: *Xylella fastidiosa*, phytosanitary plant health risk, vectors, Bulgaria

EMERGING PLANT HEALTH RISKS: CASE STUDIES FROM BULGARIA

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ABSTRACT

Phytosanitary risks can emerge as a result from a newly identified plant pest for which a significant probability of introduction and/or spread may exist. In 2011 the Panel of Plant Health at the European Food Safety Authority (EFSA) defined the following drivers of emerging plant health risks: (1) changes in pests, plants and their interactions; (2) changes in agriculture and forestry practices; (3) changes in trade, food consumption and land use; (4) climate change (EFSA, 2011). Based on EFSA material and experience from Bulgaria, the current report presents typical examples of pests as drivers of plant health risks. (1) Plant pests can cause more severe damage in recently invaded territories due to changes in their virulence, aggressiveness, host plant range, population dynamics, and other characteristic features. The occurrence of new more invasive vectors requires changes in routine control measures because the phenomenon might lead to an increase in plant diseases. Introduced species are new components at the trophic level of food chains; they may become damaging pests and quickly expand their distribution due to the absence of natural enemies at the new location. Relevant examples from Bulgaria include the species complex *Bemisia tabaci* and Tomato yellow leaf curl virus (begomoviruses), *Trialeurodes vaporariorum* and Tomato infectious chlorosis virus (criniviruses), *Frankliniella occidentalis* (Thysanoptera: Thripidae) and Tomato spotted wilt tospovirus (tospviruses), Pepino mosaic virus (PepMV) on tomato, citrus flatid planthopper - *Metcalfa pruinosa* (Hemiptera: Flatidae) and the box tree moth *Cydalima perspectalis* (Lepidoptera: Crambidae). (2) Changes in agricultural and forestry practices often lead to emergence of new and returning of old phytosanitary problems. Monocropping, periods of crop rotations and included crops, and some techniques for tillage favor the development of soil pests and pathogens. Examples are given from Bulgaria for potato wart disease (causal agent of *Synchytrium endobioticum*), potato cyst nematodes (*Globodera pallida* and *G. rostochiensis* (Heteroderidae)) and the western corn rootworm *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). The expansion of crops grown under protected cultivation contributes to the emergence and spread of tropical and subtropical pests/pathogens such as *Tuta absoluta* (Lepidoptera: Gelechiidae) and *B. tabaci* (Hemiptera: Aleyrodidae) in temperate zones. The increase of areas devoted to fresh-cut vegetable provokes emergence and reemergence of soil-borne pathogens such as formae specialis of *Fusarium oxysporum*. The planting of exotic trees in forests and green urban areas leads to the introduction of alien pests associated with them, such as the citrus long-horned beetle *Anoplophora chinensis* (Coleoptera: Cerambycidae) on Japanese maple *Acer palmatum* in Italy and the Netherlands. (3) Plants and plant products are the main pathway for introduction of new pests, and plants for planting represent the largest share. The marketing and movement of ornamentals and spices for planting is the reason for the introduction of pests such as *B. tabaci*, *F. occidentalis* and *Paysandisia archon* (Lepidoptera: Castniidae) in the EU and in the country. The trade of fruits and vegetables is not strictly regulated in the EU,

but these commodities are also an important pathway for the introduction of invasive species. For example *T. absoluta* was introduced to the EU from South America with tomato on the vine and *Drosophila suzukii* (Diptera: Drosophilidae) with infested cherry fruits from Asia. Despite the requirements of the International Standard for Phytosanitary Measures (ISPM No. 15) wooden packaging material is still a pathway for the introduction of the pine wood nematode *Bursaphelenchus xylophilus* and the Asian long-horned beetle (*Anoplophora glabripennis* (Coleoptera: Cerambycidae)) in the Community. The growing demand for novel foods, exotic and organic plant products is also a prerequisite for the emergence of phytosanitary risks. For example, propagation material of goji berries for organic production was the cause for the introduction of *Aceria kuko* (Acari: Eriophyidae) in Bulgaria in 2016. (4) Climate change is a key factor in the establishment and spread of plant pests into new areas.

Naturally occurring thermophilic pests and their vectors can expand their distribution to the north due to the elimination of climatic barriers. Rising winter temperatures will lead to the expansion of the geographical range of the oak processionary moth *Thaumetopoea processionea* (Lepidoptera: Thaumetopoeidae) in Northern Europe. In our country this species was first reported more than 100 years ago. Some pests (e.g. *Ceratitis capitata* (Diptera: Tephritidae)) and vectors (*F. occidentalis*) can overwinter in Bulgaria and cause damage and transmit viruses to plants early next year. Prediction and management strategies Predicting plant health risk requires pest monitoring and analysis of the data on pathways of introduction and interceptions. The development of models including with respect to the impact of climate change on the introduction and / or spread of plant pests is also useful. CLIMEX models reflecting the opportunities for entry and establishment in Bulgaria of the pests *D. suzukii*, *T. absoluta* and *F. occidentalis* were developed. The main strategies for management of phytosanitary risk include: a pest risk analysis (PRA) before the import of new plants and plant products; development and implementation of rapid methods for detection, identification and monitoring of pests such as diagnostic kits and indicator plants; ranking and prioritization in the presence of plant health risk; development of early warning systems in the European Union. In some European countries (the Netherlands, Germany and UK) express PRA is prepared first, to identify species that require full PRA. The rapid eradication of new invasive pests is sometimes connected to socio-economical and political issues (for example the program for eradication of the potato wart disease in Bulgaria). There is a need for development and coordination of appropriate contingency plans. The European and Mediterranean Plant Protection Organization (EPPO) has developed key elements of such a plan in case of new plant health risks. Member states (incl. Bulgaria) also have such plans, but need better coordination in the administration and implementation of actions.

Key words: *plant pests and diseases, phytosanitary emerging risks, plant health, Bulgaria*

RESEARCH ON THE POSSIBILITY OF APPLYING ENVIRONMENTAL DISINFECTANT IN THE FOOD INDUSTRY

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An environmental disinfecting product (anolyte) has been tested under laboratory conditions. The product was produced by electrochemical activation of 0.5% aqueous solution of NaCl using a high-tech equipment "STEL 6 m 11". Immediately after producing the anolyte, its main parameters have been determined, as follows: pH, concentration of active chlorine and oxidation-reduction potential (ORP). The anolyte has been tested for bactericidal activity in accordance with the Bulgarian Standard BDS EN 1040:2006. In view of the possibility for long-term use in practice, its disinfection efficiency has been monitored for a 6-month period. Based on the results of the tests performed, it is concluded that the anolyte represents a reliable disinfectant without side effects for application in the food industry.

Keywords: anolyte, disinfection, bactericidal activity

GENERAL CONSIDERATIONS OF ANIMAL DISEASE PREVENTION FOR THE BALKAN PENINSULA. THE BULGARIAN POINT OF VIEW

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ABSTRACT

The animal population of the Balkan Peninsula has been suffering seriously from the incursion of exotic viruses over the last years. Assessing the risks related to those agents it has to be noted that some of the viruses have also the potential of being a zoonotic agent and transmitted by vectors spreading very fast in time and geography not respecting any administrative or territorial border. Taking into account the observations made during the latest epidemics it seems evident that those viruses related incursions cannot be contained by just applying a stamping out policy on the hosts/susceptible animal species and accompanying eradication measures such as movement control, movement restriction of animals and desinsection of vectors. Moreover, it appears that only a vaccination strategy properly carried out is a successful tool to finally contain the spread of the diseases. The preconditions for an efficient and effective vaccination strategy on the Balkan Peninsula will be discussed taking into account the Bulgarian experience. Such prerequisites considered regarding the Balkan region are -a permanent common scientific monitoring tool for early identification of disease risks, -a targeted sampling scheme for early identification of agents, -an appropriate laboratory capacity for agent isolation and characterization with a view to develop a vaccine, -

a vaccine production facility, -a fast track procedure for testing the safety, efficiency and effectiveness of the vaccine, -a speedy system for the distribution of the vaccine, -legislation not discriminating vaccinated animals and affecting the trade negatively following successful vaccination and -a common epidemiological research tool specially focussing on the role of vectors.

ZIKA VIRUS – EMERGENCE, EVOLUTION, PATHOLOGY AND PERSPECTIVES FOR CONTROL

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ABSTRACT

Zika virus (ZIKV) belongs to the family. Flaviviridae, genus. Flavivirus and antigenically is very similar to Yellow fever, Japanese encephalitis and West Nile fever viruses. Until recently it was thought that geographic distribution of this virus is limited within the equatorial and sub-equatorial areas of Africa and Asia and is determined by the presence or absence of the relevant competent mosquito vectors such as *Aedes albopictus* (Tiger mosquito) and *Aedes aegypti* (Yellow fever mosquito). These vectors are responsible for the spread of some exotic viral diseases such as Dengue, Yellow Fever and Chikungunya.

In 2016 the World Health Organization (WHO) announced ZIKAV as a global threat. The epidemic of ZIKAV first appeared in Brazil in the spring of 2015. The virus has already spread to 62 countries and territories around the World, and is expected to rise up to 4 million cases of the disease in North and South America next year.

The disease in patients infected with ZIKAV most often is manifested with fever, rash, joint pain and conjunctivitis, although some of them develop a temporary neurological disorder called Guillain-Barre syndrome (GBS). In several northeastern states of Brazil quite often occurs microcephaly syndrome in new born babies from women already infected with the virus during the pregnancy.

The perspectives for the development of an effective vaccine against ZIKAV could be expected in one or two years period. The only way to prevent the disease is to control the mosquito populations by eliminating the places of their breeding sites (biotopes) or by carefully use of approved insecticides to maintain ecological balance in the environment.

The possibilities of some advanced methods for vector control are discussed such using male mosquitoes infected with bacteria of the genus *Wolbachia*, This bacteria leading to feminization of male mosquitoes. Other perspective approach is using the methods of genetically modified organisms (GMOs). By directly manipulating the genome of the male mosquitoes is implemented vertically transmitting and self-limiting gene leading to reduce the population to 90%.

The final conclusion is that globalization and environmental changes; social and demographic changes and the capacity of the health system are the three interacting drivers that can determine the scenario of development of epidemics of ZIKAV.

Key words: ZIKAV, *Aedes albopictus* (Tiger mosquito), *Aedes aegypti* (Yellow fever mosquito), Guillain-Barre syndrome (GBS), genetically modified organisms (GMOs), vector control, mosquito populations

BORRELIOSIS: LESS KNOWN AND UNFAMILIAR VECTOR-BORNE ZOOSES

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ABSTRACT

Provided is updated information on the two main groups of Borrelia species: the first LYME DISEASE Borrelia (well-known in humans and less familiar in animals); and the second RELAPSING FEVER Borrelia (known in humans and almost unknown in animals). Described are the epidemiological characteristics of borreliosis from the first group and in those from the second, has been focused on the new and little known in dogs and cats Borrelia turicatae, Borrelia hermsii, Borrelia persica. Examined are the difficulties in laboratory diagnostics - microscopy, cultivation, serology, PCR - in respect to accuracy and reliability of the individual methods. Analyzed are interesting case studies and the peculiarities of clinical findings, haematological tests and other clinical and laboratory characteristics in dogs, horses, cats and people. Discussed are the therapy peculiarities and the problems in the healing process. Proposals are made to raise awareness of pet owners about the risks of vector-transmitted infections. Emphasized is the role of the collaboration between human / veterinary doctors in the name of the total public health mission “One Medicine -One Health”.

Key words: Lyme Disease, Tick-borne Relapsing Fever, *Borrelia burgdorferi*, *Borrelia persica*