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LECTURES

A1

GEOPOLITICAL DETERMINANTS OF FOOD SECURITY: AN INTERDISCIPLINARY CONTRIBUTION.

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According to the United Nations Organization for Food and Agriculture (FAO) food security exists when all people, at all times, have physical and economic access to sufficient safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life. The 2014 report on world food insecurity estimates that about 805 million people are chronically hungry, i.e. one in nine persons worldwide, and generally around 2 billion suffer from the lack of nutrients or have "hidden hunger". Agricultural growth in general and advances in science and food technology in particular are needed to reduce hunger and malnutrition, but are not enough. In this sense it should be integrated into the design of local policies the broad set of macro-level variables that could respond to the geopolitical challenges facing the world in agri food issues, thus enabling better conditions for social inclusion. Particularly since the rise of food prices and volatility in 2008 as a long-term trend, certain global risk factors have come to occupy a central place in the areas of analysis and decision-making at the international level. Geopolitical factors such as global competition for natural resources, energy insecurity, population growth, ecosystems degradation and biodiversity loss, it is estimated that pose a great challenge for a safe and affordable food supply. Both regions dependent on an external supply as the large food and food commodities producers such as Argentina, are required to weigh and integrate into its strategic planning these issues of structural level and work in a co-participation at local and regional level along with the several public and private actors in the key sectors of production, processing and consumption that make the food system.

A2

FOOD ADDITIVES. LEGAL ASPECTS CONCERNING SAFETY. NEW CHALLENGES

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The use of substances to preserve food, improve safety, maintain nutritional value or enhance flavor and color has a long tradition dating back to ancient days. With the increasing use of processed foods there has been a great increase in the use of additives that led to legislation to regulate their use. Up to the moment more than 2500 different chemical compounds – natural and synthetic - have been evaluated and included in the *Codex Alimentarius* List of Food Additives. Legally, the term additive refers to "any substance the intended use of which results or may reasonably be expected to result -- directly or indirectly -- in its becoming a component or otherwise affecting the characteristics of any food." This definition includes any substance used in the production, processing, treatment, packaging, transportation or storage of food. The composition and properties of the substance; the amount that would typically be consumed; immediate and long-term health effects, and various safety factors are considered when evaluating the safety of a substance and whether it should be approved as a food additive. The evaluation determines an appropriate level of use that includes a built-in safety margin - a factor that allows for uncertainty about the levels of consumption that are expected to be harmless. In other words, the levels of use that gain approval are much lower than what would be expected to have any adverse effect. All additives are subject to ongoing safety review as scientific understanding and methods of testing continue to improve. In spite of the legal framework and regulations about the use of food additives, their safety has been and is a matter of concern among consumers. The use of *Lactic acid* bacteria to prevent the growth of pathogenic species and enzymes produced biotechnologically as additives in food industry are two new challenges.

A3 MOLECULAR GASTRONOMY

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One of the accepted meanings of the word “food” refers to all things that man and animals eat or drink to survive. The amount of time spent in food preparation and in eating, the importance of kitchen and dining room in a house design and the use of food in maintaining social relationships, suggest a direct association of foods with the pleasure of eating as well as the need to prolong life. Engineering applies science and technology in order to achieve the massiveness and safety of food products, often at the expense of quality. More recently, Molecular Gastronomy (MG) pays special attention to the enjoyment of eating by studying the physical and chemical transformations that occur during the preparation of a recipe. The recipes have definitions (ingredients and techniques) and precisions (tips, tricks and recommendations). Definitions can be modeled based on the presence of interfaces between solids, water, oil and air. Precisions can be categorized into the following groups: some that seem wrong and they are wrong (stirring creams in the same direction), some that seem wrong and they are right (best meringues are obtained in copper vessels), some that seem right and they are wrong (sealing meat to prevent loss of juices), and some that seem right and they are right (addition of lemon juice to delay enzymatic browning). The scientific contribution of MG to food service led to the manufacture of avant-garde dishes based on foams, airs, emulsions and gels using ingredients and techniques originally developed for industrial production. In that sense, our group is developing edible films based on calcium alginate gels, flavored with broth and then cooked, in order to be used as a replacement of roasted animal skins with reduced concentration of high-risk compounds (cholesterol, triglycerides, etc.).

A4 IMMUNONUTRITION

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Diet and immune system have been known to be associated to each other. Epidemiological observations have confirmed that infection and malnutrition aggravate one another. Protein-energy malnutrition is associated with a significant impairment of cell-mediated immunity, phagocyte function, complement system, secretory immunoglobulin A, secretory antibody response and cytokines production. Therefore, the deficiency of specific aminoacids provokes an impairment on humoral and cellular immunity. Up to date, several investigations were performed on glutamine and arginine immunoregulation effects. Similar findings related with impairment of immune system, have been reported for moderate or relative mild deficiencies of single nutrients such as trace minerals and vitamins, particularly Zn, Fe, Se, vitamins A, B6, C and E. For example, Zn deficiency is associated with profound impairment of cell-mediated immunity such as lymphocyte stimulation response, decreased CD4+:CD8+ cells, and decreased chemotaxis of phagocytes. Higher intakes of single nutrients also results in altered immune response.

A5 BIOLOGICAL SCIENCES IN THE CINEMA: AN APPROXIMATIVE REVIEW

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The cinema was officially invented by the French brothers Auguste (1862-1954) and Louis (1864-1948) Lumière during 1890 and patented and commercially inaugurated in 1895 through their documentary short film *Sortie de l'usine Lumière de Lyon*. However, some authors attribute its invention to the French chemist and engineer Louis Aimé Augustin Le Prince (1842-1890), who filmed some sequences entitled *Roundhay Garden Scene* in his parents-in-law's house (Leeds, England) using a single-lens camera and Eastman Kodak paper film. This technique and art has a particular relevance for

the teaching and learning processes of biological sciences and for people investigating it. In this regard, it may motivate and stimulate decisions, generate competences linked to knowledge, attitudes, habits, values and skills, disclose rivalries among researchers, promote scientific-technological dissemination, create the emulation of appropriate behaviours oriented towards Biology and encourage debates (cinema forum). In that context and excluding a lot of existing documentary films, the presentation is mainly focused in successive promotional posters recalling silent and talking biographic fictional full-length films (biopics) and talking fictional full-length films related with biological issues as evolution, bioethics, scientific investigation and method and greenhouse effect, among others. All of them are centred in the abovementioned relevance between cinema and biological sciences. Determined requirements are the established for an adequate cinematographic achievement as (a) critical thinking for avoiding exaggerations, omissions and historic mistakes, and (b) proper skills for distinguishing reality from fiction and essential from superfluous as well as for decoding the significance of the images. Different sites (articles, books, Internet, festivals, animations and documentaries) apt to be consulted for persons interested in the addressed subject are finally detailed.

A6

DIETARY STRATEGIES TO IMPROVE DYSLIPIDEMIA AND INSULIN RESISTANCE

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The prevalence of chronic illnesses like obesity, insulin resistance (IR), glucose intolerance, hypertension and dyslipidemia currently reach epidemic proportions. The causes of these diseases included in the metabolic syndrome (MS) -where IR plays a central role- are not completely elucidated, but the complex interaction between genetic, metabolic and environmental factors is well recognized. MS favors the incidence of cardiovascular disease (CVD) and type 2 diabetes. Different strategies were carried out to prevent/ improve all these risk factors. The composition of dietary macronutrients may enhance/prevent any of these disorders. Clinical and experimental studies show that dietary n-3 polyunsaturated fatty acids (PUFAs), especially EPA, 20: 5n-3 and DHA 22: 5n-3, exert a beneficial effect by regulating the levels of lipids, cardiovascular and immune function and insulin action. Moreover higher concentration of ALA, 18: 3n-3, another PUFA from plant sources, precursor of EPA and DHA is associated with a reduced risk of CVD disease by lowering dyslipidemia. These events are pursued regulating nuclear events involved in the transcription of lipid and carbohydrate metabolic genes and adipogenesis or by the incorporation of n-3PUFAs at the membrane phospholipids which in turn alter signal transduction processes. Conversely, diets rich in saturated fat or single carbohydrates are related to obesity or visceral adiposity respectively and IR, dyslipidemia and hypertension. Protein intake is also associated to the management of metabolic disorders included in the MS. Studies in animals and humans suggest that consumption of soy protein has positive effects on obesity and lipid metabolism. Consumption of soy protein can improve satiety, body fat, CVD, lipid metabolism, hypertension, glucose homeostasis and IR. In experimental animal's models, dietary soy protein and their isoflavones appear to modulate the expression of nuclear transcription factors that regulate fatty acid metabolism and cholesterol homeostasis. Finally, the use of experimental animal models that mimic the nutritional phenotype of human MS are extremely useful for further basic understanding of the management of these metabolic alterations susceptible to dietary manipulation.

A7

INFANT BOTULISM, TRANSMISSION AND PATHOGENESIS, THEIR RELATIONSHIP WITH FOOD AND POSSIBILITY OF PREVENTION BY PROBIOTICS

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Botulism (IB) is a potentially fatal disease characterized by flaccid paralysis caused by botulinum neurotoxins. In 1976 was recognized the infant IB, in babies under 1 year of age, by ingestion of *C. botulinum* (Cb), and other species, that colonize the intestine. It is the most common form of botulism today. In most cases, the origin of Cb spores could not be determined. The two recognized sources were soil (and environmental dust), and honey. The first identifications of Cb spores in herbs,

like chamomile and linden used in teas for infants, were done in Argentina. Breast feeding appears to delay the onset and the progress of IB, and reduce the risk of respiratory arrest.

The diet may be one of the most important factors that influence the composition of the normal intestinal microbiota (NIM). The dominant members vary, depending in part, on whether infants are fed only breast milk or formula or mixture of both, and if the diet includes other foods. The infant NIM contains different species of bacteria, but especially *Bifidobacterium* that may interfere *in vitro* multiplication and/or toxin formation (MTI) of Cb. Between 2012-2013, we studied the MTI of Cb by the probiotic *Lactobacillus paracasei* subsp. *paracasei* in co-culture. We observed growth inhibition in solid media, and decrease of the toxin formation in liquid media. The reduced levels of toxin (LD₅₀/mL) were very significant (up to 6 log₁₀), compared with Cb monocultures. Cb is not part of the NIM, and we are always exposed to environmental and food spores. As IB is not transmitted from person to person, the risk of colonization is restricted to the susceptibility of infants to colonization of Cb and toxin formation in the intestine. For prevention, it should be considered educational programs for the population, oriented to: decrease contact with ambient dust, hygiene, not give honey or herbal teas, breastfeeding, and consider supply pre- and/or probiotics, perhaps from the birth, in order to interfere colonization and/or toxin formation by Cb in the gut.

A8

ANTIMICROBIAL EFFECT OF *Xenophyllum poposum* ON THE ORAL MICROBIOTA RELATED TO DENTAL CARIES

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For the control of dental caries different prevention methods are used like mouthwashes with Chlorhexidine (CL) as an adjuvant of toothbrushing. There is a great interest in researching natural substances with antimicrobial effect due to microorganism's resistance to traditional drugs. *Xenophyllum poposum* is a subshrub from the northwest of Argentina and is employed in many diseases. Our research group studied the *in vitro* effect of different extracts of *Xenophyllum poposum* (Xp) prepared in hexane (EH), chloroform (EC) and ethanol (EE) on microorganisms isolated from saliva: *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus casei*, *Actinomyces naeslundii*, *Actinomyces odontolyticus*, *Candida albicans* and *Veillonella*. Taking CL as the antimicrobial of reference, the EE inhibited all strains under study producing less inhibition on *Veillonella*, which was positive considering that this microorganism is an indicator of health. Minimum Inhibitory and Minimum Bactericidal Concentration of the EE of Xp were determined on the microorganisms in study. The action of EE of Xp was evaluated by transmission electron microscopy to determine the cellular changes produced. It was found damage in the cell wall observing irregular condensations in the cytoplasm. The Xp has bactericide action on cariogenic species which suggests that this natural substance could be used to prevent this infectious disease.

SHORT COMMUNICATIONS

Facultad de Ciencias Bioquímicas y Farmacéuticas

A9

***Escherichia coli* STRAIN CARRYING A *bla*_{KPC-2}-CONTAINING CONJUGATIVE PLASMID: AN INFREQUENT CLINICAL FINDING**

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The spread of strains belonging to different *Enterobacteriaceae* species and producing acquired serine-carbapenemases like KPC, constitutes an important clinical problem. We characterize the genetic platforms containing *bla*_{KPC-2} in clinical isolates of non-*Kpn* members of the *Enterobacteriaceae*. We analyzed two *Enterobacter cloacae* (*Ecl*) complex species (*Ecc*) and 2 *Escherichia coli* (*Eco*) clinical isolates which were collected between 2013 and 2014 from patients in Rosario hospitals, all of them resistant to all β -lactams including the carbapenems. The presence of *bla*_{KPC-2} in these strains was verified by specific PCR and sequencing. Moreover, degenerate oligonucleotide-based PCR assay revealed the existence of two *Eco* clones (L and J), and a single clone (H) for *Ecl*. The presence of plasmids carrying *bla*_{KPC-2} was evaluated by plasmid purification and conjugation/ transformation assays. Plasmid purification and transformation into *E. coli* DH5 α showed only 2 strains, *Eco* clone J and *Ecl*, carried ampicillin resistance determinants. Conjugation assays revealed that the ampicillin resistance-conferring plasmid from *Eco* clone J is conjugative. Sequencing of *bla*_{KPC-2} genetic context from *Eco* clone J indicated a Tn4401 derivative. The characterized mobile platform suggests that enterobacterial species other than *Kpn* are involved in the spread of the *bla*_{KPC-2} resistance determinant in environments subjected to carbapenem selection pressure.

A10

MISSENSE MUTATIONS RESPONSIBLE FOR WEAK D ANTIGEN EXPRESSION

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The *RH* locus consists of two highly homologous genes, *RHD* and *RHCE*. These genes encode RhD and RhCE polypeptides that are erythrocyte transmembrane proteins. Multiples *RHD* alleles are responsible for aberrant expression of the D antigen (D variant phenotype), manifested by a reduced intensity of haemagglutination reactions. The aim of this work was to characterize at molecular level 3 samples with a weak D antigen expression. RhD and RhCE phenotype was analyzed using monoclonal antibodies. PCR-SSP strategies were performed and each of the 10 *RH* exons was sequenced. Serologic analysis showed an altered expression of the D antigen in the 3 samples. Besides, 2 of them expressed C antigen and the other one the E antigen. Sequence analysis revealed 3 unpublished mutations. One of the RhC+ variant carried the 763G>A punctual mutation in the exon 5 of *RHD* gene, responsible for Gly255Arg change. The other RhC+ sample had the 764G>A substitution, leading to the Gly255Glu aminoacidic replacement. In the RhE+ variant it was detected the 911T>A mutation in the exon 6 of *RHD* gene, responsible for the substitution Ile304Asn. The replacement of a polar aminoacid (Gly) for charged ones (Glu, Arg) and the change of an apolar for a polar residue may difficult the integration of the Rh polypeptides in the erythrocyte membrane. The resulting conformational modifications might be responsible for the weak D antigen expression observed. The molecular analysis of the *RH* locus is important for the development of reliable DNA characterization strategies for *RHD* prenatal genotyping and for optimizing blood units selection in Blood Banks.

A11

VASCULAR FLORA OF THE PROVINCE OF SANTA FE: CALLITRICHACEAE AND LINACEAE

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The families Callitrichaceae and Linaceae belong to the class Magnoliopsida (=Dycotiledoneae). In old classification systems (Engler, 1892) were both located in the Order *Geraniales*, but in the second half of the twentieth century, and because of their embryological characters, the Callitrichaceae were considered as belonging in Metachlamydeae. Currently, according to the APG III system, they are located in the Lamiales. On the other hand, Linaceae were excluded from Geraniales by Takhtajan (1966) and Cronquist (1981), who placed it in the new Order Linales alongside Erythroxylaceae. At present according to the APG-III system it is located in the Malpighiales, together with other families: Salicaceae and Violaceae, one of the most notable changes in classification systems. This contribution is an introduction to the knowledge of these families and their distribution in the province of Santa Fe. The methods consist of a bibliographical review, consultation of national herbaria with important collections of the province (SF: Esperanza; SI: San Isidro; UNR: Zavalla), field work experience of the authors and lab work to confirm their identity. The preliminary results show that the Callitrichaceae is represented in Santa Fe by two terrestrial species: *Callitriche deflexa* A. Braun. ex Hegelm. and *C. terrestris* Raf. subsp. *subsessilis* (Fassett) Bacigalupo, typical in low and moist soils in the north of the province. The family *Linaceae* is represented in the province by a native species: *L. littorale* A. St.-Hil. It has also been observed the spontaneous presence of *L. usitatissimum* L. on road sides. Taxonomic information, distribution map and illustrations are provided.

A12

ZINC LEVELS IN SEMINAL SPOTS AND ITS APPLICATION IN FORENSIC CRIMINOLOGY

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The prostate gland has high concentration of zinc, it is proposed detection by an analytical method in spots on a piece of cotton, which can be used as a reliable forensic marker presence of semen. The seminal plasma is the biological fluid containing the highest concentration of zinc, average value of 150 ug/ml. Our objective was to evaluate the levels of zinc in seminal spots on cotton and its application in forensic criminology. Semen samples of men between 20 and 45 years old were analyzed. Spots on pieces of cotton (n=33) were performed by applying 200 ul of seminal plasma and its allowed to dry. Fragments of 0.5 cm diameter were removed and cut areas 0.7854 cm². The same procedure was performed as a control on a piece of cotton without stains (n=33). The samples were placed in tubes and 0.4 ml sulphuric acid and 0.6 ml nitric acid were added; it was heated at 60°C for 6 hours with periodic agitation, then to a final volume of 10 ml done with distilled water. The standard solution was prepared with zinc granules and the calibration curve was constructed. The samples and dilutions of standard were processed in atomic absorption spectrophotometer. The average zinc in 33 samples analyzed was 23.47±9.09 ug/cm² and in the controls was 3.00±1.00 ug/cm². The averages zinc concentration in the samples with seminal spots on pieces of cotton were compared with controls using the *t*-student test and significant statistically difference was found (p=0.006). According to the obtained results, the analytical determination of zinc from a spot on a piece of cotton allows us to identify the presence of seminal fluid. Its application is important in cases of sexual abuses by oligospermic or azoospermic subjects where it is difficult to determine the presence of spermatozoids.

A13

EFFECT OF THE ASSOCIATION β AMYLOID -APOLIPOPROTEIN J (ApoJ) ON THE ACTIVITY OF MEMBRANE ATTACK COMPLEX (MAC) OF THE COMPLEMENT SYSTEM.

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Brain lesions that characterize Alzheimer's disease (AD), mainly composed of fibrillar aggregates of amyloid beta protein ($A\beta$) also present components of the complement system (CS) whose presence suggests an *in situ* activation process. Fibrillar and soluble $A\beta$ activate CS resulting in the generation of its activation products and MAC formation which could result in neuronal cell lysis. Another protein strongly associated with $A\beta$ is ApoJ, a chaperone protein which also acts as a regulator of C5b-9 complex (MAC) of the CS at C7 level covering lipophilic sites which are exposed as a result of activation. Its levels are increased in brain affected regions and in the cerebrospinal fluid (CSF) of patients with AD. The present work aims to study the effect of $A\beta$ -ApoJ association on the activity of the individual components of the MAC: C5, C6, C7, C8 and C9 using a hemolytic method that measured functionality of the molecules. We use pure C5, C6, C7, C8, C9 and the corresponding deficient serum in each of them (Sigma). CXH50 unit (hemolytic activity) is defined by the supplier as the amount of CX capable of lysing 50% of 3×10^7 sheep erythrocytes sensitized with antibody (EA) when incubated in the presence of 20ul of CX deficient serum for 30 minutes at 37 °C in a final volume of 500ul. Using this technique, the hemolytic activity of each individual component (3 independent experiments in duplicate) was measured in the presence of the synthetic peptide of sequence homology to $A\beta$, ApoJ pure (Quidel), the combination of both and absence of all of them. The results show i) ApoJ added to the reaction medium in concentration equal to twice the normal average concentration in CSF (3 $\mu\text{g/ml}$) inhibits 17 ± 2 % C7 activity. Its association with $A\beta_{1-40}$ neutralized in a dose dependent manner (from 3.0 ngr/ml, normal mean concentration in CSF, up 30.0 ngr/ml) such inhibitory effect; ii) $A\beta_{1-40}$ -ApoJ, at previous same concentrations and relationships, increases 20 ± 2 %, 40 ± 3 % y 8 ± 0.5 % the hemolytic activity of C5, C8 and C9 respectively. These results clearly indicate that the activating signal, $A\beta_{1-40}$, is capable of overcoming even increased regulatory mechanisms (ApoJ) and constitute a further evidence of the importance SC activation as a potential mechanism of neurotoxicity

A14

EVALUATION OF THE OCCUPATIONAL EXPOSURE TO ENVIRONMENTAL FACTORS THAT CAN ALTER THE SPERM OF INFERTILE MEN

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There are various natural or synthetic chemical compounds to be released into the environment cause damage especially un human reproductive health. The spermatogenesis is a process that develops in the seminiferous epithelium, rich medium in androgens. Our objective was to evaluate in infertile men the effect of occupational exposure to environmental risk factors on sperm parameters. The sperm study according to WHO 2010 have been carried out to 117 semen samples. The risk factors considered were: exposure to agrochemicals, toxic products and the body contact or intermittent exposure to heat. The percentage of progressive motile sperm (PM) was determined by optical microscope and thermostated plate at 37°C applying subjective method; the concentration of germinal cells (GC) was estimated with the Papanicolaou stain. The percentage of spermatozooids with functional membrane was determined with the hipoosmotic test. The concentration of seminal fructose (F) was measured using a spectrophotometric method. The relationship between variables is studied through statistical models of multiple and logistic regression. In semen samples of men exposed to agrochemicals found higher percentage of spermatozooids with decreased PM ($p=0.0495$) and concentration of F outside the reference range ($p=0.0335$). Higher concentration of GC ($p=0.0197$) was found in the semen of men in contact with sources that produce heat. In workers exposed to toxic products a decreased of spermatozooids with functional membrane was found ($p=0.0198$). The occupational exposure with environmental risk factors induces seminal alterations and should be considered when evaluating the male factor in infertile couples.

A15
GENOTYPE OF ABH ANTIGENS IN HUMAN SPERMATOZOA

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ABH glycoconjugates have been studied in genetics, anthropology, associated with various diseases and also with human infertility. Near 25% of infertile couples show reproductive failures by male factor. Sperm membrane has glycoconjugates related to recognition, cellular adhesion, signals transduction and metabolic processes that control its maturation, transport and interaction with the oocyte. Glycosyltransferases A and B are responsible of conversion of H antigen in A and B. The aim was to determine homo or heterozygote status of ABO genes using PCR to evaluate its possible association with infertility. Semen samples of 21 infertile patients and 28 voluntary fertile donors were studied according WHO criteria(2010). As a molecular strategy, genomic DNA was obtained and two pairs of primers were designed in order to amplify two different gene regions: Set I, amplifying exon 5 and Set II amplifying exon 7. PCR was carried out with a unique protocol and without using restriction enzymes. By comparison of the bands of products, the individual genotype was determined. Fertile controls presented 14 O (50.00%), 11 A (39.28%), 3B (10.7%) in agreement with normal frequencies in our population. The 21 ABO blood groups of infertile men were distributed as follow: 7 O (33.3%), 6 A (28.57%) and 8 B (38.09%), showing a high prevalence of B group ($p < 0.001$) and its probable association with infertility. We observed greater homocozis in B group than in A. This data suggest that would exist an association between genotype B and infertility. Additional studies of glycoconjugates on spermatozoa as in other cells of genital tract will provide knowledges about molecular mechanisms that control reproductive process. This strategy is accurate, simple and fast, and it could be considered in the evaluation of male infertility.

A16
MOLECULAR BASIS OF A KELL NULL PHENOTYPE (K₀)

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The *Kell* system is one of the most clinically relevant blood groups, is highly immunogenic. The antibodies are involved in the pathogenesis of hemolytic disease of the fetus and newborn and severe hemolytic transfusion reactions. The *Kell* blood group system comprises at least 34 antigens where K / k, Kp^a / Kp^b and Js^a / Js^b are the most important epitopes. The combined expression of these antigens is determined by different alleles of the gene *KELL*. The aim of this study was to characterize the molecular basis of a *KELL* null phenotype. A sample of peripheral blood of a pregnant patient alloimmune was studied. *KELL* phenotype was analyzed using monoclonal antibodies detecting the absence of expression of K / k, Kp^a / Kp^b and Js^a / Js^b antigens. To confirm these findings PCR-RFLP strategies were applied and kk-Kp^bKp^b-Js^bJs^b genotype was obtained. Because of the discrepancy between serological and molecular findings were sequenced each of the 19 exons of *KELL* and intron-exon splice region. A point mutation was identified in the first nucleotide of intron 3 (*IVS3+1g>a*). The nucleotide substitution was found located in the region of mRNA splicing. The replacement of the conserved sequence GT by AT would be responsible for an alternative assembly of mRNA with loss of exon sequences. This change would cause a frameshift generating a premature stop codon. Molecular studies have revealed that the patient's erythrocytes do not express the membrane glycoprotein *KELL*. Identification of the mutation responsible for this exceptional phenotype will allow the development of a PCR allele specific to detect in the household carriers of this allele and provide appropriate genetic counseling with regard to possible alloimmune reactions through transfusion therapies and / or pregnancy.

A17

TOXOPLASMOSIS AND PREGNANCY. TESTING FOR SEROLOGICAL DIAGNOSIS

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Toxoplasmosis is a parasite zoonosis caused by *Toxoplasma gondii*. The aim of this work was to assess the prevalence of recent infection by *T. gondii* in pregnant women. Methods: Cross-sectional study. It was performed detection of IgG and IgM antibodies anti *T. gondii*; then, the reactive samples to IgM were assayed to IgG avidity test, utilizing the same two commercial methods, ELFA (enzyme-linked fluorescent assay) and CMIA (microparticles chemiluminescent immunoassay). Serum samples were from patients of Hospital Provincial del Centenario, Rosario, who were attended between March and November, 2013. Commercial kits used were CMIA (Abbott) and Elfa (bioMérieux). Results: Using ELFA it was found (n=64): IgG: (reactive; cut-off level ≥ 8 IU/mL) 76.6% (95% CI: 64,3-86,2%); IgM: (reactive; cut-off level $\geq 0,65$) 28.1% (17,6-40,8%). IgM reactive samples (n=18) found that they had a test with low avidity 44.4% (21,5-69,2%). Using CMIA it was found (n=68): IgG: (reactive; cut-off level: $\geq 3,0$ IU/mL) 70.6% (58,3-81,0%); IgM: (reactive; cut-off level: ≥ 0.60) 27.5% (17,5-39,6%). IgM reactive samples (n=19) found that they had a test with low avidity 47,4% (24,4-71,1%). There was no significant differences between both methods for low avidity proportion (p=0.87). Conclusion: Among the IgM reactive samples approximately half presented a result of low avidity, compatible with an acute infection. There were no differences between the implemented diagnostic methods. Although results must be correlated with the clinic, it is necessary to deepen the measures of prevention of this disease, taking into account the risk associated with a primary infection during pregnancy for the normal development of the child in gestation.

A18

DEVELOPMENT OF AN ASSAY FOR HEPATITIS B VIRUS MOLECULAR SCREENING IN BLOOD DONORS

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Transmission of hepatitis B virus (HBV) infection during the serological window period is an important concern in transfusional medicine. Here we show the development of a PCR assay for the molecular detection of the HBV DNA that includes a universal internal control (UIC) to verify every analytical step, and the following steps: a fixed amount of the UIC is added to the plasma sample before the DNA extraction; amplification by multiplex PCR using biotinylated reverse primers for each target (HBV and UIC); liquid hybridization of the biotinylated amplicons with specific fluorescein-containing probes; hybrid capture into streptavidin-coated microplate wells; colorimetric detection with a monospecific anti fluorescein antibody conjugated with horseradish peroxidase, and color production measurement in a microplate reader. The assay had a detection limit of 15 copies/reaction. Preliminary results of the test specificity indicated that the assay was able to discriminate among the different infectious conditions (acute and chronic HBV patients) and healthy donors. However, the clinical specificity of the test need to be determined using a large number of samples with different HBV infectious status and statistical analysis. The definitive validation of our molecular HBV detection method will be carried out using the "Second WHO International Standard for Hepatitis B Virus DNA", available in our lab. In conclusion, this HBV test prototype provides a sensitive, low cost molecular assay that could be evaluated for its incorporation in the molecular screening of HBV infection of blood donors attending to public blood banks in Santa Fe province, Argentina.

A19

GENETIC DISEQUILIBRIUM BETWEEN *RHD* AND *RHCE* ALLELIC VARIANTS

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RHD and *RHCE* genes encode the Rh antigens, expressed on the red blood cells membrane. Multiples *RH* alleles are responsible for altered antigenic expressions. The aim of this work was to characterize the molecular background of the allele responsible for a weak expression of the D antigen and to study the familiar segregation. Samples with D aberrant expression were obtained from H1 and H2 brothers and their father (PP). RhD and RhCE phenotype was analyzed using monoclonal antibodies. PCR-SSP strategies were applied and each of the 10 *RH* exons was sequenced. The Rh phenotype was D^{var},C-,c+,E+,e- for H1 and H2, D^{var},C-,c+,E-,e+ for PP and D,C+,c+,E+,e+ for MM (mother). Due to an apparent exclusion of paternity, the E antigen was also study with another 2 monoclonals. A weak agglutination with one clone was observed indicating that the phenotype of PP was D^{var},C-,c+,E^{var},e+. Sequence analysis demonstrated the presence of a 46T>C punctual mutation responsible for the Trp16Arg aminoacidic change. PP sample presented the 697C>G, 712A>G, 733C>G and 744T>C mutations in exon 5 of the *RHcE* allele responsible for the Gln233Glu, Met238Val and Leu245Val substitutions. This study evidenced that both mutated alleles were in *cis*. The low poblational frequency of these allelic variants suggests ligament disequilibrium between them. These associations are useful for the identification of antibodies directed to high frequency Rh antigens and facilitate the transfusional compatibility in cases of rare genotypes. The presence of charged residues may difficult the membrane integration of the Rh polypeptides, leading to an aberrant expression of D and E antigens. Our results show that molecular studies represent a necessary complement for the characterization of samples with Rh variant phenotype.

A20

COMPARATIVE STUDY OF THE EFFECT OF TWO COMPOSITIONS OF THE POTATO DEXTROSA AGAR MEDIUM FOR THE IDENTIFICATION OF *Fusarium* STRAINS

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The *Fusarium* genus consists of phytopathogenic fungi affecting many economically important crops and also by endophytes of several plant species. They show metabolic versatility, producing not only mycotoxins but also metabolites with agricultural and pharmaceutical value. Due to their highly variable physiological and morphological characteristics, different taxonomical systems have been used for the identification. According to Nelson *et al.* (1983) their identification is based on the growth on Synthetic Nutrient Agar medium (SNA) for the description of the microscopic characteristics and, on Potato Dextrose Agar (PDA) for the study of the macromorphology. The use of the classic PDA (Nelson *et al.* PDAC) and of PDA supplemented with magnesium sulfate and calcium carbonate (PDAS, FAO 1985) in the identification of 32 isolated strains compatible with *Fusarium*, was compared in this study. All strains, previously developed on Sabouraud (7 days – 25 °C), were grown in duplicate on PDAC, PDAS and SNA (14 days – 25 °C). In 30 of the 32 strains the same species was found using both media, with a substantial agreement ($K=0.9243$, $p < 0.0001$). It was determined by the application of Mc Nemar test that the use of PDAS was better than PDAC for the macromorphological description of *Fusarium* isolates allowing species identification more conclusively ($p=0.0003$).

A21

HLA-DRB1 PARTICIPATION OF POLYMORPHISM IN RESPONSE TO THE TREATMENT OF HEPATITIS C

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Hepatitis C (HC) is an infectious disease of the liver caused by the Hepatitis C virus (HCV). The virus persists in most infected patients and is responsible for a wide spectrum of liver injury. Patients diagnosed with HC, develop chronic infection predisposed to cirrhosis and hepatocellular carcinoma. Has been proposed involvement of genetic factors in the development of this disease and response to treatment. Between genetic polymorphisms associated to the development and severity of infection HCV genes are involved in immune response as the HLA system. One of the treatments used today, combined pegylated interferon and ribavirin (pegIFNa/RBV). The aim of this work was to study the association between *HLA-DRB1* alleles and response to treatment pegIFNa/RBV. We studied 38 patients diagnosed with HC who attended a medical consultation or control to Gastroenterology and Hepatology Centennial Provincial Hospital Rosario, in 2012. We consider a patient responded to treatment when we see a sustained viral response (absence of HCV RNA in plasma (< 50 IU / ml) 24 weeks after completion of treatment). DNA was extracted from peripheral blood and the molecular typing of *HLA-DRB1* alleles was performed using PCRSSP. Of the 38 patients included in this study, mean age 50 years, mean age at the time of infection average 26 years and duration of infection 24 years. We note that 20 patients (53.6 %) responded to the combination treatment and 18 were non-responders (47.4 %). When comparing the frequency of *HLA-DRB1* alleles in these two groups of patients, no significant differences were found. We believe that increasing the number of patients studied, possibly let you know if the HLA system participates in the response to combined treatment with pegIFNa / RBV.

A22

STUDY OF ABH ANTIGENS EXPRESSION IN URINARY SEDIMENT CELLS OF PATIENTS WITH BLADDER TUMORS

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In malignant transformation of tumors, the A and B blood group antigens which are normally present on various body tissues are lost. The aim of this work was to study the expression of ABH antigens in urinary sediment cells of patients with bladder tumors. 48 patients with bladder tumors and 70 patients without demonstrable pathology (control group) were studied. Urinary sediment of the first morning urine, obtained by centrifugation was used. The presence of ABH antigens was investigated in the extract, using inhibition agglutination technique with antisera specific anti-A and anti-B, and lectin *Ulex europaeus* with anti-H activity. 100% (n = 70) of the control individuals ABH antigens expressed in urine sediment cells. Of the 48 patients studied, 29.3% (n = 29) the expressed and 19 (39.6%) lost reactivity of ABH antigens in the cells studied. The proportion of patients who loose ABH expression is significantly higher than in those with bladder tumors (p <0.0001). Our studies indicate that loss of ABH antigen expression is associated with malignant lesions while preserving the antigens studied indicates a slight degree of carcinoma. These studies could be used as a complementary tool for the detection and prognosis of urogenital cancer.

A23

ASYMMETRIC HYDROGENATION OF C-C DOUBLE BOND OF N-PHENYL-2,3-DIMETHYL-MALEIMIDES BY FUNGAL STRAINS

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Most chiral syntheses are traditionally achieved through chemical methods employing an ever-expanding range of chiral catalysts and reagents. Chiral products obtained through bioconversions offer the advantage of employing a renewable and natural source of reagents and avoiding the use of solvents while operating at room temperature. Large numbers of microbes can be isolated from nature as a source of inocula for bioconversions. Filamentous fungi produces a myriad of useful enzymes with different catalytic abilities, providing the ability to mediate steps in organic syntheses. With the aim of increased the structural biodiversity of N-phenyl-2,3-dimethyl-maleimides through fungal catalysis, we isolated five fungal strains from soybean. Growing cells technique was used for biotransformation. Time of addition of the organic substrate profoundly affects the yield of product because the substrate may present toxicity toward cell growth. So, addition during the late logarithmic growth phase avoids toxicity effects. After 96 hours of the inoculation, all fungal strains arrive at this phase. *Arthrimum phaeospermum*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium solani* and *Rhizopus oryzae* have demonstrated to be efficient biocatalysts for the enantioselective hydrogenation of 2,3-dimethylated-phenyl-maleimides, constituting new efficient tools for the production of chiral succinimides.

A24

HEPATOCTE TRANSPLANTATION ISOLATED FROM RAT LIVERS WITH PRENEOPLASTIC FOCI. IMPACT OF AN IMMUNOSUPPRESSANT AGENT ADMINISTRATED TO THE RECIPIENTS.

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Transplant of hepatocytes isolated from donor (D) with hepatic preneoplastic foci (PF) developed PF in the recipient (R) after 7 days posttransplant. We compare the number of PF between R not immunosuppressed (NISR) and R immunosuppressed with Cyclosporin A (CsA) (ISR), and we analyze hepatic functionality in both group. 90-day Wistar male rats were used as D and as R. D were subjected to a 2-phase model of rat hepatocarcinogenesis to induce PF. Experimental groups: ISR, R received 20 mg/kg CsA 4 days before and 1 day after transplant; NISR were not immunosuppressed; Sham (controls). R was partial hepatectomized after transplant. At days 7, 21 and 60 R were anesthetized and bled to measure ALP, GOT and GPT. Pieces of liver were used for an immunohistochemistry using rGSTPi to identify PF. For each R the total area of tissue obtained was calculated with image software and PF were counted (n° PF/cm² of tissue). 60-day R were used to calculate the n° of PF. ALP was statistically higher for NISR and for ISR than SHAM at 7 and 21 days; TGP was higher for NISR only at 7 days, GOT did not present differences between groups or time. The number of PF was significant higher for ISR at 60 days. We concluded that the number of foci increased after 60 days in ISR recipients and that hepatic function was conserved.

A25

AFIBRINOGENEMIA, A CASE REPORT

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Circulating fibrinogen (Fg) is a glycoprotein involved in the final step of the coagulation pathway as a precursor of fibrin monomers that participate in the formation of the haemostatic plug. In July 2012, a 24-yr-old patient was admitted at the Hematology Department in Hospital Provincial del Centenario, with a clinical history of bleeding since childhood. Laboratory tests confirmed diagnosis of afibrinogenemia. The patient was submitted a posteriori to a dental extraction, and previously administered a single dose of Fibrinogen Concentrate, 50 mg/kg b.w. (Haemocomplettan® CSLBehring). The objective of the present work was to assess the time to attain Fg haemostatic levels and evaluate Fg's concentration with two methods (functional, Clauss method and antigenic, Radial Immuno Diffusion, RID method) during the patient follow up. Results: RID vs. Clauss. At 0 time: 0.0mg/dl vs. 0.0mg/dl. At 15 min: 111.9mg/dl vs. 55mg/dl. At 60min: 139mg/dl vs. 50mg/dl. At 180min: 167.8mg/dl vs. 50mg/dl. At 24h: 129.8mg/dl vs. 50mg/dl. At 72h: 103.3mg/dl vs. 31mg/dl. 9 days: 33.5mg/dl vs. 0.0mg/dl. The results demonstrated that the haemostatic level was attained at 15 min post-infusion of 50 mg/kg of Fibrinogen and was stabilized and maintained for 72h in accordance with the plasma protein half-life. On day 9, Fg was undetectable by the functional method, but a small amount was measured by RID.

A26

JAK2V617F ALLELE BURDEN (AB) IN PATIENTS WITH PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (MPNs)

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MPNs are clonal hematopoietic disorders. The most common MPNs are essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF). Many of these patients carry the JAK2V617F activating mutation, that can be harbored in the heterozygous or homozygous state, the latter deriving from mitotic recombination; homozygosity for this mutation is found in some PV and MF patients but it is rare in ET. The gene-dosage hypothesis, postulates a correlation between disease phenotype and the proportion of JAK2V617F mutant alleles. The aim of the study was to quantify the AB of JAK2V617F and correlate it with hematologic and clinical phenotype. We studied 75 patients JAK2V617F: 32 with PV, 29 with TE and 14 with MF. We obtained genomic DNA from patients at diagnosis or those without cytoreductive treatment. The AB analysis was performed with the use of two allele specific, quantitative PCR assays to measure either the wild type or mutant allele. The AB was expressed as the percentage of JAK2V617F on total JAK2. We found that the JAK2 (V617F) AB is significantly higher in patients with PV (69%) than in those with ET (38%) ($p=0.0002$) and is significantly higher in patients with MF (76%) than in those with ET (38%) ($p=0.0001$). We analyzed correlations between AB and hematologic parameters. We found a moderate and direct relationship between AB and leukocytes count and a moderate and inverse relationship between AB and platelets count ($p<0.0005$). Our results allow us to conclude that AB has influence on phenotype because we found significant differences in AB within MPNs. A higher burden of JAK2 (V617F) induces enhanced myelopoiesis and leukocytosis. Thrombopoiesis is stimulated by low AB, as an inverse relationship between AB and platelet count has been found.

A27

ASCOCHYTA BLIGHT: ISOLATION, IDENTIFICATION AND DEVELOPMENT OF A BIOAUTOGRAPHIC METHOD TO DETECT *Ascochyta rabiei* INHIBITORS.

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Ascochyta blight is the major disease that affects chickpea (*Cicer arietinum*, Fabaceae) and is caused by the fungus *A. rabiei* Pass. Labrousse. Blight significantly reduces seed yield and quality. The aim of this work was the isolation of *A. rabiei* from infected chickpeas and the development of a bioautographic method to detect natural inhibitors in complex mixtures, as new and less toxic alternatives to conventional antifungals. Infected chickpeas were surface decontaminated and plated in a medium containing chickpea extract 20% (MCG). Plates were incubated under photoperiod (12 hours light /dark). Colonies with a morphology suggesting *Ascochyta* sp. were sub-cultured to assess their macro and micromorphology, and were identified by molecular methods by means of ITS sequencing. In order to optimize the bioautographic method, four variables were evaluated: inoculum size (5×10^3 , 5×10^4 and 1×10^5 spores/ml), culture media (Sabouraud Glucose, SbG, and MCG), agar concentration (0.6%, 1% and 1.5%) and addition or not of Phenol Red as a contrast. Dipheniconazole and Chlorothalonil were used as positive controls and were spotted over thin layer chromatography (TLC) plates, which were then covered by warmed culture media containing the inoculum in a final volume of 0.1 ml/cm². After 48 h incubation, a viability staining reagent (MTT 1 mg/mL) was added to visualize inhibition halos. Optimal conditions for bioautographic assay resulted in the use of SbG 0.6% agar plus Phenol Red and inoculum size of 1×10^5 spores/ml. This methodology will allow us to search for natural inhibitors in plant and microorganism extracts.

A28

Candida STRAINS BIOFILM PRODUCTION AND SUSCEPTIBILITY TO PHOTSENSITIZER AGENT TOLUIDINE BLUE O

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Photodynamic therapy has emerged for the treatment of infections produced by microbial pathogens, including them capable of forming biofilms. The objective of this work was to study the biofilm production by different *Candida* strains and the effect of photodynamic therapy with Toluidine Blue O (TBO) over them. Twenty *Candida* strains were isolated as causative of oropharyngeal candidiasis (*C. albicans* N=8, *C. tropicalis* N=4, *C. parapsilosis* N=5, *C. krusei* N=2, *C. glabrata* N=1). In the first stage, quantitative biofilm measurement was performed in a 96-well microtiter plate. Briefly, yeast were grown and incubated for 24 h at room temperature, then cultures were poured out, washed and stained with crystal violet solution. After solubilization with ethanol-acetone, OD_{595 nm} was determined. Strains were noted as strong biofilm producers when OD ≥ 0,24; strains with OD between 0,12 and 0,24 were designated as moderate producers and those with OD < 0,12 as no producers. In the second stage, antifungal photosensitive activity against biofilms were determined by the Clinical and Laboratory Standards broth microdilution technique (M27-A₃). TBO photosensitizer agent was evaluated at a final concentration of 500 µg/ml and was irradiated for 60 min. Results showed that 50% of strains were biofilm producers (25% were strong producers and 25%, moderate producers). TBO showed no antifungal photosensitive activity against biofilms.

A29

MODIFIED RESISTOGRAM METHOD FOR THE STUDY OF YEAST FROM ORAL MUCOSA OF CANCER AND NON-CANCER PATIENTS WITH CANDIDIASIS, AND HEALTHY VOLUNTEERS

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Genus *Candida* is part of microbiota in human skin and mucous membranes. However, it can cause opportunistic infections in immunocompromised patients, such as those under oncological treatment. The ability to invade and antifungal resistance promote the development of infections. The aim of this study was to compare characteristics of *Candida albicans* strains, isolated from oral cavity in three study groups: cancer patients with radiotherapy in head and neck (N=31), non-cancer patients with candidiasis (N=48), and healthy volunteers (N=45). They were cultured in Sabouraud agar supplemented with sodium selenite (A), boric acid (B), cetrimide (C), sodium periodate (D), malachite green (F) and copper sulfate (G) (modified resistogram method). Growth in the different media was recorded, being for example [ABCDFG] an isolates resistant to all compounds, and [A---G], a resistant isolate to boric acid and copper sulphate. Frequency of *C.albicans* profiles were not the same in different groups (p <0,0001- Fisher Test). [ABCDFG] profile was found more frequently in cancer patients than in non-cancer, while [ABC-F-] was more frequent in non-cancer patients with candidiasis than in those with cancer. Profiles [A-CDFG] and [A-C-F-] were present only in isolates from cancer patients, [ABC-FG] and [A---FG] exclusively in non-cancer patients; eventually, [---DF-], [-B-DF-], [-B-DFG] and [--CDF-] only in strains from healthy volunteers. Due to the high frequency of oral candidiasis in patients under cancer treatment, it is important to deepen the studies in order to understand the behavior of the genus, to correlate the resistance to chemicals with the aggressiveness of microorganisms, to be able to assess the need for appropriate prophylaxis to reduce risks of invasion and infection.

A30

SYNTHESIS AND ELECTRONIC SPECTRUM (UV-VIS) OF TERNARY COMPLEXES OF SULFA DRUGS WITH THE COBALT(II) ION

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Sulfonamides were the first effective therapeutic agents for the prevention and the cure of bacterial infections in human. The formation of complexes among metal ions and sulphonamides constitute a wide field of investigation since the sulfas, as their metal complexes possess a wide spectrum of actions, not only the antibacterial. In this work, we report the synthesis of three new ternary complexes of Co(II) with sulfa drugs and as a second ligand, DL-Methionine (Met) and o-Phenanthroline (Phen). Sodium sulfathiazole (NaST) and ftalilsulfathiazole (H₂FST) were the employed sulfa drugs, which their homoleptic cobalt(II) complexes, that have shown antifungal properties, have been already obtained and characterized in our laboratory. Water's solutions have been mixed containing 0,5 mmol of the reagents (the sulfonamide, Co(NO₃)₂·6H₂O and the second ligand: Met; Fen –in ethanol-). The obtained precipitates were washed 3 times with water, centrifuged and let them dry at room temperature in the dark. Elemental chemical analyses (C, H, N, S), which were performed in a CARLO ERBA EA 1108 microanalyser, gave satisfactory results for: CoC₂₃H₂₈N₇O₇S₅:Co(ST)₂Met(H₂O); CoC₃₀H₂₆N₈O₅S₄:Co(ST)₂Fen(H₂O) and CoC₃₀H₂₈N₅O₁₀S₂:Co(FST)(Fen)·5H₂O. Electronic spectra of the obtained complexes were recorded in DMSO (λ: 300-800 nm, spectrophotometer Jasco V-550, double beam, 25 °C). Spectra of ternary complexes with Fen showed shifts towards lower wavelengths, suggesting a higher energy crystal field splitting due to the presence of Fen, a strong ligand field.

A31

IDENTIFICATION, CHEMICAL PROFILES AND BIOACTIVITY OF *Fusarium* ENDOPHYTIC STRAINS

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Fungal secondary metabolites play an important role in the discovery and development of new therapeutic drugs. Endophytic fungi are a diverse group that live inside plant tissues without causing disease, and represent an important source of new bioactive chemical entities. We have previously isolated 64 endophytic fungi from aerial parts of *Peperomia obtusifolia* (L.) A. Dietr. (Piperaceae). The aim of this work was to perform a morphological and molecular identification of 7 fungal isolates compatible with the *Fusarium* genus, to study their metabolic profile in potato dextrose agar (PDA) media with the addition of MgSO₄ and CaCO₃, and to analyze the antibiotic activity of their extracts. Two isolates were identified as *F. oxysporum* by analysis of their micro and macro morphology in PDA and Synthetic Nitrogen Agar, following classical taxonomic keys, meanwhile the five left were compatible with *F. oxysporum* but also with *F. solani* and *F. subglutinans*; and were identified by means of the Internal Transcribed Spacer (ITS) sequencing as *F. oxysporum*. All strains were sub cultured in PDA for 15 days at 28 °C in dark, colonies were extracted with ethyl acetate and extracts were analyzed by thin layer chromatography and Mass spectrometry by direct infusion (negative mode). The *m/z* signals for each isolate were compared by Principal Component Analysis (PCA). The 3 first PC showed PO4 to PO7 as a cluster, while PO1, 2 and 3 were separated from each other. Almost all extracts showed antibiotic activity against *Staphylococcus aureus* and *Mycobacterium smegmatis* while *Escherichia coli* was only inhibited by PO2. The great metabolic variation existing among isolates shows the importance of the use of chemometric tools, in addition to chemical and bioactivity assays, in the screening for strains producing interesting secondary metabolites.

A32

S100 A9, A PROTEIN PRESENT IN HUMAN OVIDUCT, BINDS TO GAMETES *IN VITRO*.

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We have identified S100 A9 protein in human oviduct secretion. Our aim was to investigate whether S100 A9 binds to oocyte zona pellucida (ZP) and to sperm. Human oocytes were collected from donors and were incubated with or without 10 µg/ml of human recombinant S100 A9. Motile sperm from normozoospermic donors (WHO criteria) were incubated in capacitating conditions with or without 10 µg/ml of S100 A9, for 1 h or 6 h at 37 °C and 5% pCO₂. Acrosome reaction (AR) was induced with progesterone and was detected by fluorescein isothiocyanate-*Pisum sativum*. Detection of bound S100 A9 was performed with anti-S100 A9 and Cy3-anti-IgG conjugate. Four groups were analysed: 1) sperm cells incubated 1 h in capacitating conditions (non-capacitated); 2) cells incubated for 6 h in capacitating conditions; 3) cells incubated in capacitating conditions for 6.5 h that did not undergo AR; 4) cells incubated in capacitating conditions for 6.5 h that underwent AR. Cells were counted in an epifluorescence microscope. Results were expressed as mean percentage ± standard error medium. Oocyte ZP analysis revealed a fluorescent signal confirming S100 A9 binding. Percentage of spermatozoa that showed S100 A9 bound in groups 1 and 2 were 9.4 ± 1.0% and 13.4 ± 2.7%, respectively (p = 0.18, n = 9). Moreover, S100 A9 bound to 6.3 ± 2.1% and 17.0 ± 2.5% of sperm from groups 3 and 4, respectively (p = 0.02; n = 3). In conclusion, S100 A9 bound to human ZP and to spermatozoa. We showed that the protein binds to a small sperm population which mildly increases after incubating in capacitating conditions. Results indicated a significant increase in S100 A9 binding to acrosome-reacted sperm, suggesting that this protein may be involved in the modulation of this process.

A33

THE FRACTAL SLOPE: A NONLINEAR APPROACH TO STUDY THE DESHIALIZATION CAUSED BY MUSCULAR LARVAE OF TRICHINELLA SPIRALIS

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The *Trichinella spiralis* parasite, is a pathogen that caused the trichinosis, which infected form is the cyst of tricchina. The cellular mechanisms that appears during this process are not well known, but it was communicated that on one hand would participate the glucoconjugated from the parasite and the host and on the other previous experiences had shown that the muscular larvae captured the sialic acid, this suggest the possibility that the helmint could take it from the muscular cells. In this work we studied the erythrocytes aggregation produced by the in vitro contact between red blood cells (GR) and muscular larvae, through the Fractal Slope (D), using digital image processing, with free software FRACTALYSE. To obtain D we followed an iterative process, making a zoom on the image details. We analysed mathematically step level vs. the number of occupied spaces. On answering the main object of this work, we used GR type O as model cells, which were incubated in equal parts with the larvae concentrated. We work with 6 GR control samples, and larvae concentrations at 4500-5000 larvae/mL y 8500-9000 larvae/mL, at time in minutes: T1=0, T2=60, T3=120, in 4 experiences, obtained images by duplicated. The data obtained was normalized and no normalized, choosing the first one that offers better discrimination power. The fractal slope results: a) constant for control GR: $D_0=1.49\pm 0.19$, $D_{60}=1.44\pm 0.22$, $D_{120}=1.49\pm 0.24$, While for GR-larvae at T2, appears an increase of 10% and T3 a decreased of 7%. This would show the larvae interaction with GR, produced a change on the fractal patterns which appears as a change on D. These preliminary results will be completed with new experiences at intermediate times, to provide consistency to the results.

A34

ASSOCIATION OF ANTI-*Saccharomyces cereviceae* ANTIBODIES WITH SEX, AGE AND INTESTINAL BIOPSY IN CELIAC PATIENTS. PRELIMINARY STUDY.

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Celiac disease (CD) is an intolerance to gluten suffered by genetically susceptible individuals. It is characterized by inflammation, villi atrophy and hyperplasia of crypts in the small intestine. The mechanism by which gluten peptides cross the intestinal epithelium remains elusive, but an altered intestinal permeability has been proposed to play a crucial role in the pathogenesis of CD. Studies about the presence of anti-*Saccharomyces cerevisiae* antibodies (ASCA) in patients with Crohn's disease suggest that ASCA are markers of the integrity of the intestinal mucosa, although their role in the pathogenesis of this disease is not fully known. Moreover, it is generally accepted that *Saccharomyces cerevisiae* is not a pathogen, but an ubiquitous yeast normally used in the manufacture of a wide variety of foodstuffs. Our aim was to analyze the association between ASCA and sex, age and intestinal biopsy in celiac patients. We studied 15 pediatric patients, from the Gastroenterology Service of the Hospital de Niños V. J. Vilela. The levels of ASCA IgG/IgA in serum were determined by ELISA. The intestinal biopsy was evaluated by means of the histological classification of Marsh-Oberhuber. No statistically significant differences ($p>0.05$) were found between ASCA and sex, age and intestinal biopsy, but it is worth noting that ASCA-IgG was more frequently found in male patients than in female ones (33% vs 17%) and in ≤ 5 -year-old patients than in > 5 -year-old ones (33% vs 22%). Eighty percent of the patients with subtotal atrophy presented a negative result. ASCA-IgA was found negative in all patients studied. Our results suggest that the IgG isotype of ASCA could be relevant in the evaluation of the integrity of the intestinal mucosa in CD.

A35

**ASSOCIATION OF BIOCHEMICAL MARKERS OF CELIAC DISEASE WITH SEX, AGE AND
INTESTINAL BIOPSY IN CHILDREN. PRELIMINARY STUDY.**

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Celiac disease (CD) is an intestinal autoimmune disorder characterized by intolerance to gluten. Our aim was to analyze the association of the following antibodies (Ab): anti-tissue transglutaminase IgA (a-TGt IgA), anti-endomysial IgA (EMA IgA), anti-gliadin IgA/IgG (AGA IgA/IgG) and anti-deaminated gliadin peptide IgG (a-DGP IgG) with sex, age and intestinal biopsy in 15 pediatric patients with CD. The a-TGt IgA, AGA IgA/IgG and a-DGP IgG were assessed by ELISA and EMA IgA by IFI. The intestinal biopsy was evaluated by the histological classification of Marsh-Oberhuber. AGA-IgG levels were significantly higher in ≤ 5 -year-old patients than in older ones ($p=0.044$). No statistically significant differences ($p>0.05$) were found for all the other biochemical parameters studied, although it is worth noting that: a) AGA-IgA was more frequently found in female patients than in male ones (67% vs 44%), in ≤ 5 -year-old patients than in older ones (83% vs 33%) and in those with normal biopsy (NB) or infiltrative biopsy (IB) than in those with atrophy (A) (100% vs 42%); b) AGA-IgG was more frequently found in patients with NB or IB than in those with A (100% vs 58%); c) In males and in ≤ 5 -year-old patients a high degree of positivity of EMA-IgA was observed (100% and 83%); patients with NB, IB or partial A had a low or moderate degree of positivity, while 60% of patients with subtotal A showed a high degree of positivity; d) Titers of a-DGP-IgG > 50 U/ml were found in 78% of males, in 83% of ≤ 5 -year-old patients and only in patients with a-DGP A; e) 89% of males and 67% of females showed titers of a-TGt IgA >76 U/ml, while only in children > 5 years old and in patients with a-TGt IgA titers < 50 U/ml were found. Since patients with NB showed elevated levels of Ab, the need rises for a re-consideration of the biopsy as the “gold standard” in the diagnosis of CD.

Facultad de Ciencias Médicas

A36

**PLASMA CHOLESTEROL INCREASE IN RATS WITH MODIFIED LIPID ENRICHED DIETS.
BLOOD FLUIDITY ANALYSIS.**

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To obtain an increase of plasma cholesterol in rats to study the effect of different hypocholesterolemic agents, the animals were fed with two different diets analyzing the factors acting upon blood fluidity. Male Wistar rats aged 70 days ($n=24$), were allocated to 3 groups and fed during 28 days as follows: 1) Standard diet (C); 2) Standard diet added (D1) with 15% bovine juice (100g: 1.2g Cho, 1.06g total lipids and 6.8g proteins), and 3) Standard diet added (D2) with 0.8g 97% pure Cho and 28% wheat oil (W/W). The rats were anesthetized with sodium pentobarbital (50 mg/kg b.w., i.p.) to draw blood by cardiac puncture. Plasma assays: Cho, HDL-Cho, and LDL-Cho. Blood assays: total blood viscosity (TBV) and plasma viscosity (PV). Standardized relative BV (SBV) at 45% hematocrit $[(BV/PV)^{45/Hto}]$; rigidity index (RI) the inverse of erythrocyte deformability (ED); cell shape and the corresponding morphological index (MI). Statistics: ANOVA and post hoc Tukey's test. Results: (mean \pm SD): Cho (mg %): C:85.99 \pm 3.29; D1:77.17 \pm 2.61; D2:104.87 \pm 2.39**; HDL-Cho (mg%): C:56.17 \pm 4.12; D1:66.14 \pm 3.23; D2:79.12 \pm 1.57**; LDL-Cho (mg%): C:13.99 \pm 0.89; D1:13.67 \pm 0.67; D2:34.25 \pm 1.982**; SBV: C:4.26 \pm 0.9; D1:4.85 \pm 0.41; D2:6.56 \pm 0.18**. RI: C: 5.79 \pm 0.18; D1:6.06 \pm 0.17; D2:8.77 \pm 0.31** MI: C: -0.49 \pm 0.04; D1: -1.397 \pm 0.11**; D2: -2.458 \pm 0.12**; (** $p<0.001$ vs. C). Only D2 showed an increase of plasma Cho, HDL-Cho, and LDL-Cho, and an increase of SBV due to a decrease of ED, therefore an IR increase. The diminution of the MI points out the existence of lower ED coincident with a SBV. The increase of Cho in plasma leads to a higher relation Cho/phospholipids membrane diminishing the red blood cells deformability

A37

THE RELATIONSHIP BETWEEN ANTHROPOMETRIC INDICATORS OF ABDOMINAL OBESITY RELATED TO CLINICAL USE AND SERUM LEVELS OF 25 (OH) D.

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Abdominal obesity and hypotavinosis D pose a threat for health because of their numerous associations with cardiovascular disease and metabolic problems. The aim of this work was to use the waist/size index as a tool for abdominal obesity evaluation in patients with vitamin D metabolism possible alterations. 96 women post-menopausal were studied. Age, 25(OH)D, PTH, iCa, TG, HDL, indexes: waist-to-height ratio (WHtR); waist-hip ratio (WHR); waist-thigh ratio(WTR); waist circumference (WC); body mass index (BMI) and body adiposity index (BAI) were evaluated. The sample was divided in Group 1: WHtR<0,5(n:26) and Group 2: WHtR ≥0,5 (n:70). Results: (Average±DS and statistic meaning (p) of Group 1vs Group 2). Age: 64±10; 62±9, ns; BMI: 23,07±2,66; 30, 46±4,65, p<0,001; BAI: 31,05±4,68; 36,42±5,19, p<0,001; 25(OH)D: 27,00 ±11,13; 20,55±6,55, p<0,001; WC: 72,71±5,18; 89,27±11,01, p<0,001; WTR: 1,65±0,16; 1,76±0,18, p<0,001; WHR: 0,77±0,05; 0,83±0,06,p<0,01; TG: 94±40;130±62, p<0,001; HDL: 73±21; 61±15, p<0,01. The levels of PTH and iCa were found within the expected value range for both groups. Regarding group N° 1, in Group 2 were observed; a higher value of anthropometric indexes, a higher level of serum of TG and lower serum concentration of HDL and 25 (OH) D. The results obtained, would indicate that the anthropometric indexes used in this work, are a helpful and simple tool for the evaluation of abdominal obesity, an anomaly related to cardiovascular risk and insufficient levels of vitamin D.

A38

STUDIES OF BILIARY SECRETION IN HYPERCHOLESTEROLEMIC RATS TREATED FOR THREE AND TEN DAYS WITH PROANTHOCYANIDIN EXTRACTED FROM *Ligaria cuneifolia-Lc*

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We analyzes the effect on the biliary excretion of BS in hypercholesterolemic rats treated daily with a dose of PLc during 3 or 10 days consecutively. Male Wistar rats of 70 days of age (n = 24), were fed for 28 days with a standard diet supplemented with 0.8g / 100g of Cho (97% purity) and 28% (w / w) of corn oil. At the end, the animals were injected ip with saline (control group: C) or 3 mg / 100g body weight with PLc (T) during 3 and 10 days every 24 h . One day after the last injection rats were anesthetized with sodium pentobarbital (50mg / kg body weight, ip) and bile was obtained by cannulation of the bile duct, collecting 10 min for 60 min. After, blood was obtained by cardiac puncture and euthanasia was performed by excess anesthesia. Determination: Cho total plasma in blood, bile flow (BF), concentration of bile salts [BS]. Output of bile salts (BS) was calculated. The groups C3 and C10 showed no significant differences, whereby a single group control are presented. The results, expressed as mean ± SE, were analyzed with analysis of variance followed by Tukey post-test: Cho Total (mg%): C: 119.74 ± 1.26; T3: 61.18 ± 3.30 *; T10: 68.50 ± 1.86 *; BF (ul / min.g liver): C: 1.42 ± 0.06; T3: 1.90 ± 0.04 *; T10: 1.92 ± 0.07 *; BS (nmol / min.g liver): C: 41.36 ± 3.35; T3: 69.05 ± 3.07 *; T10: 63.18 ± 3.74 *; (* P <0.05 vs C). In rats hypercholesterolemic, the PLc -treatment during 3 or 10 days, led to a similar decrease in the total Cho by increased biliary excretion of BS.

A39

ERYTHROCYTE LIPID PEROXIDATION IN HYPERTENSIVE PATIENTS

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The aim of the present study was to analyze lipid peroxidation (LP) and LDL-Cholesterol (LDL-Chol) in 40 hypertensive patients and 40 healthy controls (without positive family history) and the correlation of both variables in hypertensive patients. LDL-Chol was calculated by Friedewald equation: $LDL\text{-Chol} = Total\text{-Chol} - (HDL\text{-Chol} + TG/5)$, where TG is for Triglycerides. Patients with TG above 300mg were not included in the study. Total-Chol, HDL-Chol and TG determinations were performed by an automated standardized method Roche-Hitachi COBAS c 501. TBARS technique was used for LP assay and results were expressed as malondialdehyde (MDA/ml of packed erythrocytes). Hypertensive patients presented significantly higher values of lipid peroxidation (nmol MDA/ml of packed erythrocytes: 4.07 ± 1.26 vs. 2.95 ± 0.61 $p < 0.01$) and LDL-Chol (mg/dl: 146 ± 27 vs. 102 ± 21 $p < 0.01$), regarding controls. Besides, there was also observed a significant correlation between MDA and LDL-Chol in hypertensive patients ($r = 0.46$ $p < 0.005$). The results of the present work demonstrate that, in the hypertensive patients, the LDL-Chol values are moderately but significantly augmented regarding controls and that LDL-Chol particles are exposed to a microenvironment with higher levels of oxygen reactive species, consequently more vulnerable to oxidation.

A40

CARRIAGE OF *Staphylococcus aureus* IN MEDICAL STUDENTS AT THE NATIONAL UNIVERSITY OF ROSARIO.

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Staphylococcus aureus (SA) is one of the most important pathogen causing community and nosocomial infections. SA is transmitted primarily through direct person-to-person contact especially through the hands of health care workers. A nasal carrier often contaminates his/her own hands by hand to nose contact, then transmits the organism in the course of routine activities to other individuals.

The aim of this study was to determine the percentage of carriers, and identify methicillin sensitive and methicillin resistant *Staphylococcus aureus* in a medical-student group. This contributes to the control of nosocomial transmission as well as to control the transmission outside the hospital.

A total of 233 medical students were recruited. Specimens were taken from nasal swabs, the samples were cultured onto manitol-salt agar. For bacterial identification we were used conventional method and susceptibility testing was performed using Kirby Bauer's disc diffusion method.

Of 233 students, 59 were positive for SA: 55 SAMS and 4 SAMR. These are preliminary results, but the presence of SA in a quarter of the population studied encourages us to continue in the quest to identify healthy carriers helping to reduce the spread of this bacteria.

This presentation is part of an ongoing research project.

A41

NASAL CARRIAGE OF *Staphylococcus aureus* (SA): RESISTANCE TO MACROLIDES AND LINCOSAMIDES.

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Nasal carriage of SA has been increasing, as well as resistant to methicillin (MRSA) strains. One alternative therapeutic is macrolides, lincosamides and streptogramin (MLS). The most frequent MLS-resistant phenotypes are MLSb (methylase) constitutive (cMLSb) and inducible (iMLSb), and MSb (efflux). The aim of this study was to determine the frequency of resistance to erythromycin and clindamycin SA isolates from nasal swabs among medical students at UNR. The collected samples were cultured onto mannitol salt agar. Identification was performed by conventional tests. To determine the antimicrobial susceptibility Kirby Bauer method was applied in accordance with Clinical and Laboratory Standards Institute (CLSI). For quality control strain ATCC 25923 was used. A total of 233 samples were processed, SA was isolated in 60 (25.75%), of which 4 were found to be MRSA. Regarding resistance MLS group, only two showed cMLSb SA isolates iMLSb 15 (25% of SA isolates). According to these results erythromycin and clindamycin would not be a valid alternative to empirical treatment of infections SA. We must continue working towards a greater number of isolates SA.

A42

NASAL CARRIAGE OF METHICILLIN RESISTANT *Staphylococcus aureus* (SA) COMMUNITY ACQUIRED (CA-MRSA) IN THIRD YEAR STUDENTS OF MEDICINE AT THE NATIONAL UNIVERSITY OF ROSARIO (UNR).

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SA colonizes the skin and nasal passages of healthy people and produces lots of infections. There is an increased incidence of infections with unusual severity due to CA-MRSA, but the rate of colonization of the population is unknown in our country.

The purpose of this study was to determine the nasal carriage of CA-MRSA in third year medical students of UNR.

A total of 111 students were screened taking a sample of nasal swabbing. The specimens were cultured on mannitol salt agar.

The identification was done using conventional tests and the antimicrobial susceptibility by the diffusion method according to standards of Institute clinical and laboratory (CLSI).

In 28 samples (25%) were recovered SA, 26 were methicillin sensitive and 2 (7%) were CA-MRSA. No hospital strains of MRSA were isolated.

The determination of this microorganism in nostrils of healthy population is useful to prevent horizontal transmission to others and severe infections in the carrier. Decolonization topical antimicrobial is a therapeutic option for carriers, but can re-colonize.

The responsible use of antimicrobials would reduce the morbidity and would limit the emergence of resistant strains.

A43

PROTECTIVE EFFECT OF QUERCETIN AGAINST *in vitro* ERYTHROCYTE RHEOLOGY ALTERATIONS PRODUCED BY ALUMINIUM

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We observe that aluminium (Al) *in vitro* damaged human erythrocyte (HE) deformability (ED), aggregability (AG), shape cell (SC) and osmotic fragility (OF) through oxidative damage. Quercetine (Qc) is well-known antioxidant and it has the ability to keep a reduced cellular medium. In this work, we study the possible protective effect of Qc against *in vitro* erythrocyte rheology alterations produced by Al. Washed HE were incubated as follow: I) in PBS, pH: 7.4 10⁷ (control); II)

in 3 μ M Qc solution, 10' (Qc); III) in Qc solution, 10' and later in AlCl₃ solution (1 μ M), 30'(Qc-Al); IV) in Al solution, 30' (Al); determining: (i) rigidity index: (RI) (high RI low ED) by filtration through pores of 5 μ m; ii) AG by optical method estimating: T (the size of the aggregates) and V (the aggregation rate); iii) SC by morphologic index (MI) (microscopic), iv) OF photometrically at 540 nm, with a X₅₀ value (NaCl mM concentration yielding 50% hemolysis). Statistic Analysis: ANOVA; values are presented as mean \pm SD RI :I) 9.45 \pm 2.48 (n:17)^{a,b}, II)9.14 \pm 2.66(n:15)^{a,b},III) 8.02 \pm 1.34(n:12)^{a,b},IV)14.17 \pm 0.63(n:14)^b.OF(X50mM(n:10)):I)68.65 \pm 2.20^{a,b},II)66.51 \pm 2.67^{a,b}, III)69.43 \pm 1.74^{a,b},IV)81.84 \pm 2.35^b. SC(IM):I)-0.074 \pm 0.04(n:10)^{a,b}, II) 0.058 \pm 0.072 (n:10)^{a,b}, III)0.256 \pm 0.194(n:10)^{a,b}, IV) 0.685 \pm 0.22 (n :14)^b. AC:T: I)1.86 \pm 0.05(n:42)^{a,c}, II) 1.87 \pm 0.03(n:31)^{a,c},III)1.86 \pm 0.05(n:37)^{a,c}, IV)1.81 \pm 0.05(n:35)^c; V:I)1.33 \pm 0.50 (n:42)^{a,c}, II) 1.20 \pm 0.45(n:31)^{a,c}, III)1.30 \pm 0.51(n:37)^{a,c}, IV) 0.77 \pm 0.26(n:35)^c (a;ns;b;p<0.05;c: p<0.005). These results indicate that Qc prevents injure caused by Al, probably through antioxidative action. In this way, the flavonoid allows that red blood cells to preserve their mechanical properties in presence of Al

A44

EVALUATION OF THE CONSUMER OF DISACCHARIDES WITH AND WITHOUT REDUCING POWER IN UNIVERSITY STUDENTS.

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Disaccharides are part of carbohydrates which are biomolecules composed of carbon, hydrogen and oxygen and provide immediate and structural energy to the body. They are classified according to the carbonyl group that it composes. The disaccharides of biological interest are: sucrose-glucose + fructose without reducing power, and lactose-glucose + galactose and maltose -glucose + glucose, with reducing power. The aim of this study was to determine the consumption of disaccharides with and without reducing power in university students. Material and Methods: An anonymous survey of 215 university students in the health area belonging to Public Universities both state and private, between 17 and 41 years old, of which 156 were female and 59 were male, asking about the consumption of refined sugar - sucrose-, banana – sucrose-, sweet potato – maltose- and whole milk -lactose- was conducted. Results: 139 women choose refined sugar and 17 do not consume it; 134 women consume banana and 22 do not; 87 women consume sweet potato and 69 do not; 96 women consume whole milk and 60 do not. Considering the male sex, 56 men choose refined sugar and 3 do not; 50 men consume banana and 9 do not; 41 men consume sweet potato and 18 do not; 39 men consume whole milk and 17 do not. It was concluded that both women and men students consume more quantity of food without reducing power. It is relevant to point up and promote the importance and knowledge of the consumption of this reducing power food.

A45

CONSUMPTION OF TRACE ELEMENTS IRON AND COPPER IN UNIVERSITY STUDENTS

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Trace elements are bioelements present in small amounts (less than 0.05%) in living organisms. Both its absence as its excess can be harmful to the body. Among these, there are the iron (Fe) and the copper (Cu), which are essential because the organism can not produce them and must get them from the environment, especially from food. The recommended daily intake of Cu according to the Institute of Medicine of the USA is 0.90mg / day in over 18 years old. In relation to Fe, 18 mg / day is recommended in women and 8 mg / day in men. The aim of this study was to know the consumption of both elements in university students. So, a cross-sectional study was done through surveys with open and closed questions, answered anonymously and voluntarily by university students from both state and private ones. 215 people were surveyed in an age range of 17-41 years old; 156 were women and 59 men. Data were grouped according to sex and to the consumption of the following products, source of Cu and Fe: beef (Cu 0.25mg / 100g and Fe 2.5mg / 100g), egg (Cu 0.74mg / 100g and Fe 2.2mg / 100g), and lentil (Cu 0.79mg / 100g and Fe 7.1mg / 100g). 92.30% of Women consumed beef and 7.69% did not; 87.18% consumed egg and 12.82% did not; the 56.41% ate lentils and 43.59% did not. Instead, 98.31% of Men chose beef and 1.69% did not; 93.22% chose egg and 6.78% did not; 54.24% ate lentils and 47.76% did not. It was concluded that the two most consumed products in both sexes were beef and egg. It is observed an early and healthy food education with respect to the incorporation of these essential trace elements.

A46

EFFECT OF FLUORIDE IN DRINKING WATER ON GROWTH PLATE CARTILAGE IN RATS

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The growth of long bones occurs in the epiphyseal growth plate and the endochondral ossification process can be disturbed by chemical factors. The toxicity of fluoride (F) is a health problem that affects millions of people worldwide. The aim of this study was to evaluate the effect of different doses of sodium fluoride (NaF) administered in drinking water on the process of endochondral ossification in growing rats. Twenty-one-day old Sprague-Dawley rats were divided into 3 experimental groups of 6 rats each and were given 0 (NaF0), 15 (NaF15) and 25 (NaF25) ppm of fluoridated water. After 30 days, left tibiae were removed and its length was measured. Longitudinal histological sections of the metaphysis were stained with hematoxylin-eosin and growth plate was analyzed. The thickness of growth plate (GPC.Th) and reserve (RZ.Th), proliferative (PZ.Th) and hypertrophic zones (HtZ.Th) were measured. Results are expressed as mean \pm standard error and differences with respect to control were considered significant if $p < 0.05$ (*). The comparisons were performed using Kruskal-Wallis and Dunn's post test. Results: no significant changes were observed in GPC.Th although a significant increase in PZ.Th with treatment was observed: NaF0: $81.8 \pm 3.89 \mu\text{m}$, NaF15: 110 ± 18.8 , NaF25: 112 ± 4.27 * $p < 0.05$ vs NaF0). The HtZ.Th significantly decreased with the highest dose (NaF0: $118 \pm 10.2 \mu\text{m}$, NaF15: 138 ± 4.84 , NaF25: 95.5 ± 6.35 *, $p < 0.05$). The RZ.Th decreased with the dose but not significantly. The length of the treated tibiae did not differ from that of controls. The results suggest that the F chronically administered in the drinking water did not produce alterations in linear bone growth. However, F alters the endochondral ossification process increasing the proliferative layer and decreasing the hypertrophic zone of the growth plate. It remains to assess whether the altered endochondral ossification process affects the characteristics of the new bone formed and its mechanical competence.

A47

FACTORS INFLUENCING THE ACTION OF ZOLEDRONATE ON TRABECULAR BONE IN GROWING RATS

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Bisphosphonates (BP) are drugs that inhibit bone resorption widely used in children and adolescent in pathologies with low bone mass and fragility fractures. Zoledronate (Z) is the more potent BP but its use in children is cause of concern because there are not enough studies about its efficacy and safety. The aim of this study was to determine the significant influence of different factors on the action of Z on bone mass and mechanical properties of trabecular bone in growing rats. The factors studied and their levels were: dose of Z (D: Z 0-2.5-12.5-25 mg/kg bw/week subcutaneously), time of treatment (T: 15 and 30 days) and sex (S: male and female). A full factorial design 4x2x2 was applied. Sixteen-21 days old Sprague-Dawley rats were assigned to a different combination of levels of each factor. At the end of the experiment, euthanasia of animals was performed. Tibiae were used to determine the % of trabecular bone volume (% BV/TV) by histomorphometry and bone mineral density (BMD) by X-ray absorption. A compression test was performed to evaluate the fracture force (FF) on femurs. The ANOVA test was used to evaluate if the factors and its interactions were significant, assuming $p < 0.05$. The results show that T significantly influence BMD and % BV/TV (T30 > T15, $p < 0.05$). The D also influenced both variables ($p < 0.05$) but no interaction were observed between the studied factors. Regarding the FF, it was significantly influenced by the three studied factors (T30 > T15, D0 > D2.5 > D12.5 > D25, $f > m$, $p < 0.05$). The studied variables were modified as expected with the growth of the animal. The growing bone positively responds to the administration of Z in a dose-dependent manner by increasing BMD, trabecular bone volume and fracture force. These preliminary findings show that the use of Z during growth does not affect bone in an adversely manner.

A48

EFFECTS OF ALCOHOL INTAKE ON BONE STRENGTH IN β RATS

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Osteopenia and deterioration of bone microarchitecture in chronic alcoholics are associated with high risk of bone fracture. The effect of ethanol intake on bone strength was evaluated in 70-day-old β male rats (n= 4), which were given for 2 months, 3.25g of ethanol/kg of body weight/day through an orogastric catheter (group O). Seven control rats (C) received placebo (water). After euthanasia femurs were extracted and biomechanical parameters of three-bending point test at the level of the diaphysis were measured: cross sectional moment of inertia, fracture load, maximum load, stiffness, toughness, stress and Young's modulus. At the level of the distal epiphysis a compression test was performed and parameters were measured: fracture load, stiffness, toughness and Young's modulus. Data are expressed as mean \pm standard deviation and comparison between groups was performed with Student t test, differences were considered significant if $p < 0.05$ (*). There was no differences in strength at the level of diaphysis. However a slight increase in Young's modulus and a decrease in cross sectional moment of inertia were observed. As a consequence of the opposite effects on these parameters, the strength at the level of the diaphysis was unchanged. At the level of epiphysis there was a significant decrease in toughness C: 10.90 ± 4.58 mJ, O: 6.088 ± 1.18 mJ (*). Deficiency in bone architecture resulting from ethanol intake would add another reason to discourage the consumption of alcohol, particularly in individuals still growing as adolescents.

A49

EFFECTS OF ALCOHOL INTAKE ON THE OBESE SYNDROME OF β RATS

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The main characteristic of fatty liver disease is steatosis. The effects of alcohol intake on this pathology have been described. Patients with alcoholic fatty liver disease (AFLD) often present signs and symptoms of hepatic-cellular insufficiency. The objective of this study was to evaluate the effect of a daily alcohol intake on the obese syndrome of IIMb/ β rats. Biomass, feed intake, abdominal fat pads weight, plasmatic and liver lipids levels were evaluated. Male 70 days old β rats (n=7) (O) received during 2 months a daily dose of 3.25g/kg body weight of ethanol as a 13% ethanol/water solution through an oro-gastric catheterization. Control group (T) (n=7) received water. Rats were fed the usual laboratory animal diet. At the end of the experiment animals were euthanized, abdominal fat pads (PAP) (PAR) and livers were excised, relative weights were calculated as percentage of body weight. Blood and liver triacylglycerols (TAG), total cholesterol (COL) and fractions were quantified. Results: mean \pm SD. Biomass (g): T: 380.6 ± 25.84 vs O: 344.8 ± 15.06 ($p < 0.05$). Total feed intake (g): T: 1789 ± 101.2 vs O: 1642 ± 174.0 (ns). Feed energy intake (kcal): T: 6061 ± 342.9 vs O: 5561 ± 589.8 (ns). Total energy intake (kcal): T: 6061 ± 342.9 vs O: 5944 ± 589.8 (ns). Efficiency: T: 7.96 ± 1.07 vs O: 7.20 ± 0.62 (ns). PAP: T: 2.15 ± 0.25 vs O: 1.92 ± 0.24 (ns). PAR: T: 2.82 ± 0.45 vs O: 2.51 ± 0.16 (ns). Col (mg/dl): T: 127.4 ± 7.6 vs O: 122.8 ± 15.5 (ns). HDL-COL (mg/ dl): T: 39.0 ± 1.8 vs O: 38.3 ± 5.7 (ns). LDL-Col (mg/dl): T: 30.4 ± 13.8 vs O: 39.5 ± 8.5 (ns). Plasmatic TAG (mg/dl): T: 289.7 ± 73.7 vs O: 247.3 ± 78.4 (ns). Hepatic Col (mg %): T: 105.9 ± 17.1 vs O: 104.9 ± 14.3 (ns). Hepatic TAG (mg %): T: 383.1 ± 68.5 vs O: 872.0 ± 99.7 ($p < 0.001$). Energetic intakes did not differ, although O reduced its feed intake. The energetic input from alcohol -7% of total caloric intake– may be responsible for the lower final biomass in O. Hepatic TAG showed the characteristic high levels of AFLD.

A50

MURINOMETRIC PARAMETERS EVALUATION IN β RATS FED A RICH OMEGA - 3 FATTY ACIDS DIET

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Obesity is a complex multifactorial disease characterized by excessive accumulation of fat that may impair health. Genetic background and diet condition the onset of the obese syndrome and its comorbidities. The aim of this study was to evaluate the effect of a diet rich in unsaturated fatty acids on murinometric parameters of β line rats. Male 70 days old β rats fed during 3 months isocaloric and isolipidic diets -prepared as recommended by the American Institute of Nutrition 1993 (AIN 93)- that only differed in their lipid composition: diet I (control): AIN 93 (n = 7) and diet II: AIN 93 with omega-3 fatty acids (n = 7). At the end of the experiment the following parameters were measured or calculated (results were analyzed with Student t test and are expressed as mean \pm SD): body weight (g): I: 425.0 \pm 39.4 vs II: 412.5 \pm 23.2 p>0.05; tail length (from the anus to the distal tip, cm): I: 17.98 \pm 0.75 vs II: 17.79 \pm 0.72 p> 0.05; naso-anal length (cm): I: 24.48 \pm 1.36 vs II: 24.26 \pm 0.80 p>0.05; abdominal circumference (cm): I: 17.78 \pm 1.47 vs II: 17.76 \pm 0.71 p>0.05; chest circumference (cm): I: 14.65 \pm 0.29 vs II: 14.33 \pm 0.43 p> 0.05; Lee index = $\sqrt[3]{\text{body weight} / \text{naso-anal length}}$: I: 0.31 \pm 0.01 vs II: 0.31 \pm 0.01 p>0.05; body mass index (BMI)= body weight / naso-anal length²: I: 144.7 \pm 14.89 vs II: 139.5 \pm 7.10 p> 0.05. After animals were sacrificed by an overdose of sodium pentobarbital, retroperitoneal and perigonadal fat pads were excised and relative weights were calculated as percentage of body weight: I: 3.85 \pm 1.30 vs II: 4.05 \pm 0.38 p>0.05; I: 3.10 \pm 0.39 vs II: 3.13 \pm 0.31 p>0.05. The cumulative food intake during treatment did not differ between groups. In β rats consumption of diets with different quality of fat did not change neither biomass nor murinometric parameters.

A51

IMMUNE-ENDOCRINE Deregulation AND INCREASED PPAR EXPRESSION IN PULMONARY TUBERCULOSIS

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Tuberculosis (TB) constitutes a major health problem as 1/3 of the world population is infected with *M. tuberculosis*. Cellular Immune response (INF- γ and TNF- α) is both implied in infection control and tissue damage. We have found that TB patients had elevated plasma levels of proinflammatory mediators and cortisol together with diminished concentrations of DHEA, DHEA-S and body mass index (BMI). The peroxisome proliferator-activated receptors (PPAR) are implicated in the modulation of immune-endocrine-metabolic status comprising 3 isoforms: PPAR α , PPAR β/δ and PPAR γ . Since mycobacterial infection leads to increased expression and activation of PPAR γ , we investigated the expression of the 3 isoforms by real time PCR in blood mononuclear cells of pulmonary TB patients (n=8, untreated HIV-); and 7 healthy controls (HCo). Plasma levels of IFN- γ were measured by EIA, C reactive protein -CRP- by turbidimetry; whereas cortisol and DHEA-S by electrochemiluminescence. PPAR γ transcript was increased in TB patients [median (25-75%)] [9,48 (6,06-26,23)] respect HCo [2,76 (0,86-4,50)] p<0,01. Patients also showed high levels of IFN- γ [23,34 (15,90-28,72)] vs. HCo [3,46 (1,82-10,48), p<0,01], CRP (p<0,01) and cortisol (p<0,05), with decreased DHEA-S levels [89,09 (69,55-117,00)] vs. HCo [172,70 (122,10- 206,60)] p<0,05 and BMI, TB [21,20 (19,60- 26,90)] vs. HCo [28,73 (27,04- 34,60)] p< 0,05. Results suggest a link between increased expression of PPAR γ and immune-endocrine disturbance of TB patients.

A52

INTOLERANCE TO GLUCOSE AND MURINOMETRICS MEASURES RELATION IN DIABETIC RATS

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Animal models are an essential tool for studying the pathogenesis of diabetes and possible palliative. The aim of this study was to identify differences in murinometric parameters in rats submit glucose intolerance or did not. A cross-sectional study was conducted in rats of both sexes of the spontaneously diabetic eSMT line. According to the value of blood glucose after 120 min of oral glucose tolerance test, two groups were constructed (I: glucose intolerant and < 120 ; N: no glucose intolerant ≥ 120 mg / dl). Abdominal circumference (CA) and thoracic circumference (CT) were registered. Lee index (LI: BW/NL) and the BMI (BW/N) were calculated. Results expressed Mean \pm SD: Females: CA: I (14.5 \pm 0.7) vs N(14.0 \pm 1.2) p=0.318; CT: I (11.0 \pm 0.3)vs N(11.8 \pm 0.8) p=0.001; BMI: I(0.47 \pm 0.05)vs N(0.48 \pm 0.11) p=0.389; LI: I (0.28 \pm 0.01) vs N (0.28 \pm 0.02) p=0.785. Males: CA: I (16.9 \pm 3.0) vs N(17.1 \pm 3.0) p=0.930; CT: I (14.4 \pm 2.4)vs N(14.5 \pm 2.9) p=0.964; BMI: I(0.29 \pm 0.01)vs N(0.29 \pm 0.02) p=0.527; LI: I (0.29 \pm 0.01) vs N (0.29 \pm 0.02) p=0.725.

Except CT in females, no differences among groups in both sexes were detected. LI reached values close to 0.30 considered to indicate threshold excess fat corporal. BMI values were in the range from .45 to .68 that is considered normal for adult Wistar rats. The measures considered are not useful for discrimination of animals with and without glucose intolerance.

A53

PERIPHERAL EOSINOPHILS (Eo) IN CBI-IGE FEMALE MICE SUSCEPTIBLE AND RESISTANT TO *Trichinella spiralis* (Ts) AND ITS RECIPROCAL CROSSES, REINFECTED WITH THE PARASITE

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Peripheral and tissue eosinophilia are typical in trichinellosis. This process results from the activation of T helper cells type 2. The *in vivo* role of Eo in host defense against Ts is still controversial; they can either be protective or induce a pathological response. To examine whether changes in Eo observed during reinfection are associated with the magnitude of muscle parasite load (MPL), female CBI/L (resistant) and CBI+ (susceptible) mice and their F1 reciprocal crosses (n=8 per group and date of sacrifice) were infected and re-infected at 33 ± 3 days of the initial infection with two L1 larvae/g body weight. Eo were determined 2-3 days before challenge with Ts (basal), at 30 days post-infection (p-i) and at 3, 6, 13 and 30 days post-reinfection (p-ri). CBI+ and CBI/L basal Eo did not differ from the values at 30 days p-i ($P > 0.05$) and were higher in F1 ($P < 0.05$). Changes in these leukocytes levels were similar in the four genetic groups during the period analyzed. There was a decrease on day 3 p-ri ($P < 0.05$) and an early recovery (day 6 p-ri), with a peak at 13 days p-ri. These results show that genetically different hosts, that respond differently against infection with Ts, resistant CBI/L with low MPL, susceptible CBI+ with high MPL and their crosses, intermediate with predominance towards a resistant profile, modify the Eo alike. The lack of association between the values of peripheral Eo and MPL suggests a different Eo parasitocidal activity in each genotype that may be due to differences in the amount or quality of specific antibodies. Therefore, its role in protection is highly specific for each host-parasite combination.

A54

VARIATION IN INTERLEUKIN (IL) SERUM LEVELS DURING THE PRIMARY INFECTION WITH *Trichinella spiralis* (Ts) IN SUSCEPTIBLE AND RESISTANT CBI-IGE MICE

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Trichinella spiralis is the causative agent of trichinellosis, a zoonotic disease of worldwide importance. This nematode has a complex life cycle in which the same host harbors different morphological forms of the parasite: infective, adult male and female, and newborn larvae. These forms, depending on the stage of the parasite cycle, dwell in intra or extracellular spaces. Each stage is capable of differently stimulating the host immune system whose response may be further modulated by the parasite. The aim of this work was to study the variation in serum levels of Th1 (IL-2 and IFN- γ), and Th2 (IL-4 and IL-10) interleukins during a primary infection with Ts in susceptible and resistant mice. Mice of both sexes of lines CBI/C and CBI/L from the CBI-IGE colony (n=10 per sex and genotype) infected with 2 Ts L1 larvae/g body weight were used. Interleukins were determined by ELISA at 3, 6, 13 and 30 days post-infection (p-i). IL-2 showed very low values in both lines, below the detection limit of the technique. At 3 days p-i no significant differences between genotypes were observed in IFN- γ . CBI/L showed higher levels of IL-4 (P=0.03) and IL-10 (P=0.04) compared to CBI/C. Although IFN- γ and IL-4 at 6, 13 and 30 days p-i were lower in CBI/L than in CBI/C, the ratio IFN- γ /IL-4 was also lower. IL-10, involved in the polarization of the response towards Th2, did not change in the period studied but was significantly higher in CBI/L on days 3 and 6 p-i (P<0.05). CBI/L showed predominance of type Th2 associated IL, which protects the host, a result that partly explains resistance in this genotype. Thus, genotypic influences on the type and magnitude of the host immune response, which limits the duration of the intestinal phase of a primary infection, are part of the wide range of factors that determine the parasite fate.

A55

VARIATION IN SERUM LEVELS OF TH1/TH2 CYTOKINES DURING PRIMARY INFECTION WITH *Trichinella spiralis* (Ts) IN CBI-IGE MICE WITH DIFFERENT SUSCEPTIBILITY TO THE PARASITE

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Trichinella spiralis, as most parasites, modulates the host immune response for survival and can induce a mixed Th1/Th2 immune response with an initial Th1 and a subsequent Th2 predominant response. The aim of this work was to study the variation in serum levels of Th1 (IL-2 and IFN- γ), and Th2 (IL-4 and IL-10) interleukins during a primary infection with Ts, in two CBI/IGE lines with different susceptibility to the parasite. Male and female CBI+ (extremely susceptible) and CBI- (intermediate susceptibility) mice (n = 10 per sex and genotype) were infected with 2 Ts L1 larvae and serum IL-2, IFN- γ , IL-4 and IL-10 were measured by ELISA at 3, 6, 13 and 30 days post-infection (p-i). Interleukins did not differ between sexes within genotypes (P> 0.05), so they were analyzed together. IL-2 values were below the detection limit of the technique except in CBI+ 3 days p-i (8 pg / ml). From day 6 p-i CBI+ significantly increased IFN- γ and IL-4 (P<0.05), the latter to a lesser extent, so that the relationship IFN- γ /IL-4 was always greater than 2. This genotype decreased IL-10 at 30 days p-i (P<0.05). CBI- did not show any significant change in IL levels during the infection (P>0.05) but the values of IFN- γ were always higher than those of IL-4. As IFN- γ was always higher than IL-4 in both genotypes, the IFN- γ /IL-4 ratio would direct the response towards Th1 favoring chronic infection. This response determines the differences in degree of infection achieved by each host, along with other genetic factors that regulate the innate and acquired responses.

Facultad de Ciencias Agrarias

A56

**PHENOTYPIC CHARACTERIZATION OF METSULFURON-METHYL RESISTANCE
CONFERRED BY *Ahas11-4* ALLELE IN SUNFLOWER**

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The *Ahas11-4* allele confers broad-range resistance to AHAS-inhibiting herbicides and has been found in a wild sunflower population. This resistance trait was introduced into cultivated sunflower through conventional breeding methods. *Ahas11-1* and *Ahas11-3* alleles are being used for the production of sunflower hybrids with resistance to imidazolinones herbicides. The objective of this study was to characterize the level of sulfonylurea resistance of *Ahas11-4* allele using a soil-less bioassay. Six sunflower isolines containing different combinations of *Ahas11-4*, *Ahas11-1* and *ahas11* (wild type) alleles at the *Ahas11* locus were used in this study. Achenes were planted on plastic pots filled with perlite and watered with nutritive solution and different concentrations of metsulfuron-methyl. For each treatment 3 replications of 12 plantlets each were incubated under controlled conditions for 7 days. Hypocotyl length, primary root length, length of primary root with lateral roots greater than 5 mm and the longest lateral root length were measured from scanned images using ImageJ and SmartRoot. Dose-response curves and GR50 (herbicide concentration that reduces the response by 50%) were estimated using R software and *drc* package. The *Ahas11-4Ahas11-4* and *Ahas11-4Ahas11-1* genotypes showed the highest values of GR50 for all the evaluated variables and differed significantly from the other genotypes. It is concluded that *Ahas11-4* allele confers a high level of metsulfuron-methyl resistance.

A57

EVALUATION OF THE INCIDENCE AND SEVERITY OF *Fusarium graminearum* IN MAIZE (*Zea mays*) RESULTING FROM PRODUCTION FIELDS, SUBJECTED TO DIFFERENT AGRONOMIC PRACTICES

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The aim of this research was to evaluate *F. graminearum* incidence and severity in maize resulting from fields subjected to different agronomic practices, which includes planting date (FS) and selection of genotype (EG). Samples belonged to fields from small-scale pork producers from South of Santa Fe Province. In 2013/14, after physiological maturity, four samples of 25 ears were collected from each field. They were evaluated for *F. graminearum* incidence (INC) and severity (SEV) according to Reid index et al. (1999). INC and SEV were evaluated by Kruskal–Wallis one-way analysis. Significant differences were observed in INC for six evaluated fields. FS INC means on 03/12 and 12/11 were the ones that presented the highest values. FS SEV on 03/12 and 12/11 significantly differed of the rest. When selection of genotypes INC was analyzed, differential behavior was observed. The same occurred in SEV. Tijereta 680 hybrid presented the highest INC and SEV, followed by Dekalb 747, although the behavior of the later was different related to FS. The application of FS which expose the maize to different types of stress condition (environmental and/or biotic), added to susceptible EG, increase the agronomic exposure risk to this pathogen. It is needed to continue adjusting this and others agronomic practices, such as plant density and nitrogen fertilization, in order to reduce these risks.

A58

***Chloris gayana* IN SALINE-ALKALINE SOILS OF SOUTHERN SANTA FE: SEEDLING EMERGENCY WITH DIFFERENT SOIL SURFACE COVERAGES**

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Implantation of *Chloris gayana* in Southern Santa Fe has some difficulties such as planting time, type of soil where it is sown and seed size, which makes it have to be very careful in preparing the seedbed. The objective of this study was to evaluate the effect of soil cover on the emergency of *C. gayana* in an halomorphic soil. The experiment was carried out in Faculty of Agronomy, National University of Rosario, on a Typic Natracalf soil. There, three sites where the natural vegetation covering the soil on 100 % (T1), 70 % (T2) and 0 % (T3) were selected. Glyphosate was applied to eliminate previous vegetation and thickness of each coverage level was measured. On January 30, 2013, *C. gayana* cultivars Pioneer, Katambora, Top Cut, Fine Cut, Santana and Reclaimer were sown. The number of seedlings were counted and the percentage of emergence was calculated, 55 days after sowing. The results showed significant differences in the interaction between soil cover and the emergence of cultivars. The highest percentage of emergency (72 %) was achieved by Katambora in T2. Top Cut and Pioneer presented a good percentage of emergency in T2 (46% and 40%, respectively). In without coverage treatment Pioneer had higher emergency (34 %) than the other cultivars. In T1, Fine Cut (37 %) and Top Cut (35 %) showed good emergency in relation to the rest of the cultivars. In general, soil cover, soil surface moisture and photoblastic property of grass seed, allowed to achieve a good number of seedlings in all cultivars.

A59

INFLUENCE OF THREE SOWING DATES IN THE EMERGENCY OF *Chloris gayana* Kunth IN SALINE ALKALINE ENVIRONMENT IN SOUTH OF SANTA FE

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Changes in sowing date modify germination response, emergence and seedling installation of *Chloris gayana*. The objective of this work was to study the effect of three sowing dates on the emergence of three cultivars of *C. gayana*. The experience was realized in Faculty of Agronomy, National University of Rosario, in a poorly drained sector (Zavalla). The planting date were: 11/12/12 (PD1), 26/12/12 (PD2) and 01/30/13 (PD3). At every opportunity, Pioneer, Top Cut and Reclaimer cultivars (cv) were sown. Drainfall and temperatures were recorded throughout the study. The number of seedlings per plot (35-40 days) was counted and the emergence was expressed as percentage (%). Experimental design was randomized blocks with three replications. The data were analyzed by ANOVA and means were compared by Tukey test ($P < 0.05$). The rains in the 35 days after PD1 exceeded the historical average by 157%, while in PD2 and PD3 were lower by 38% and 22.8%, respectively. A strong interaction FS*cv was determined. The highest percentage of emergency were achieved by Top Cut in PD1 (57.7%) and Pioneer in PD3 (53.3%) and lowest in Pioneer in PD2 (14.7%) and Reclaimer in PD1 (17.3%) and PD2 (19%). The more uniform distribution of rainfall would explain the best emergency of Reclaimer after PD3 in comparison with the remaining dates of sowing of this cv. The high variability in summer rainfall and the characteristics of each cultivar exert a significant effect on the emergence of *C. gayana*.

A60

YIELD COMPONENTS OF SOYBEAN ISOGENIC LINES WITH DIFFERENT LEAFLET SHAPE AND SEED NUMBER PER POD.

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Seed yield (Y , $\text{g}\cdot\text{m}^{-2}$) and its components: seed number (SN, $\text{seed}\cdot\text{m}^{-2}$), pod number (PN, $\text{pod}\cdot\text{m}^{-2}$), seed per pod (SPP, $\text{seed}\cdot\text{pod}^{-1}$) and seed weight (SW, $\text{mg}\cdot\text{seed}^{-1}$) were evaluated in three pairs of soybean near isogenic lines. Each pair was composed by the FV2-L, FV9-L and FV15-L lines, with narrow leaflet and high number of four-seeded pods (64 to 73%), and their respective counterparts FV2-O, FV9-O and FV15-O lines, with wide leaflet and low number of four-seeded pods (5 to 6%). A field experiment was conducted during the 2013/2014 growing season in Zavalla, Argentina (Lat.: $33^{\circ} 01' \text{ S}$). Two plant densities (PD) were used: 20 $\text{plants}\cdot\text{m}^{-2}$ (LD) and 40 $\text{plants}\cdot\text{m}^{-2}$ (HD). Experimental design was a randomized complete block with three replications. There was interaction among lines, leaflet shape and PD ($P < 0.01$). SW was affected by PD, leaflet shape and line ($P < 0.01$). The Y was strongly associated with SN ($R^2 = 0.81$) but poorly associated with SW ($R^2 = 0.12$). SN was a function of PN and SPP. PN was higher in wide than in narrow leaflet lines ($P < 0.05$), meanwhile SPP was higher in narrow than in wide leaflet lines ($P < 0.01$). On a whole, narrow leaflet lines had 28% more SPP and 9% more SN than wide leaflet lines. The highest Y was for the line FV15-L in LD ($653.7 \text{ g}\cdot\text{m}^{-2}$), due to its high SN ($4188 \text{ seed}\cdot\text{m}^{-2}$). The high SN of the FV 15-L line was a consequence of its high SPP (3.7) and high PN ($1318 \text{ pod}\cdot\text{m}^{-2}$). This study showed that it is possible to increase SN and Y by selecting for high SPP.

A61

RELATIONS BETWEEN SOIL PHYSICAL PARAMETERS AND VEGETATION PROPERTIES IN A GRAZED PASTURE

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The objective was to characterize the interrelation between vegetation variables and soil quality physical indicators in an implanted pasture. The study was done at the Experimental Farm of Facultad de Ciencias Agrarias, UNR, in a grazed pasture sown in April 2012 composed of *Medicago sativa* L., *Festuca arundinacea* Schreb. and *Bromus catharticus* Vahl. In autumn (2014), the following variables were evaluated: abundance, grassland basal cover, aerial dry matter, litter dry matter (Br), depth of initiation of B horizon, penetration resistance of the layers 0-5 cm (PR 0-5) y 5-20 cm (PR 5-20), structural stability of 0-5 cm and 5-20 cm, visual determination of the structural quality (0-25) cm and cultural profile index (0-25 cm). Eight study areas of about 20 m^2 each were included, in which three recording sites were chosen, resulting in a total of 24 data sets, analyzed by Principal Component Analysis, using PC-ORD. For the selection of CP the randomization test included in the program was used ($p \leq 0.05$). The two first CP explained on the whole 56,50 % of the total variance (CP1: eigenvalue = 3.712, $p \leq 0.001$ and CP2: eigenvalue = 2.503 and $p \leq 0.001$). In general, PR 0-5 was higher than PR 5-20; which is associated to a greater surface densification, probably due to cattle trampling. The variables under study showed a relation between themselves in variable degree, standing out the soil physical parameters over vegetation parameters to explain the behavior of the soil-pasture complex. Br was the vegetal variable which contributed the most to the model, showing high negative correlations with both RP. This might indicate that Br exerts an important protection against compactant agents.

A62

THE *Myrtaceae* FAMILY IN THE PROVINCE OF SANTA FE (ARGENTINA)

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The objective of this work is to begin the taxonomic study and the geographical and ecological distribution of the *Myrtaceae*, in the province of Santa Fe, Argentina. This family is placed in the order *Myrtales*; it consists of 3000 species of tropical and subtropical distribution. It divides in two Subfamilies: *Myrtoideae*, with opposite leaves and berry fruit that includes all the American's species; and *Leptospermoideae*, with alternate leaves and capsular fruit, where the Australian species are placed. In Argentina there are 23 native genera with 67 species, two of which are endemic, and two subsynchronous species of the Australian genus *Eucalyptus* L'Herit. These are trees, shrubs or perennial herbs with typical subepidermic glands. Leaves simple, entire. Perfect actinomorphic flowers, 4-5-(6) merous, solitary or in simple or compound inflorescence. Calyx modified in a calyptrate structure. Corolla with white petals. Stamens numerous. Inferior ovary, 2-4 locular, 2-ovules. Hypanthium persistent or not. We worked with specimens preserved in the herbaria UNR (Rosario), SF (Esperanza) and SI (Darwinion, San Isidro); together with bibliographical revision. Our preliminary results show that the family is represented in Santa Fe by six genera and eight species: *Blepharocalyx salicifolius* O. Berg, *Eugenia repanda* O. Berg, *E. uniflora* L., *Hexachlamys edulis* Kausel & D. Legrand, *Myrcia selloi* (Spreng.) N. Silveira, *Myrcianthes cisplatensis* O. Berg, *M. pungens* D. Legrand and *Psidium kennedyanum* Morong. Taxonomic information, illustrations and a distribution map are provided.

A63

GROWTH, CHLOROSIS, AND AHAS ACTIVITY ASSESSMENT IN IMAZETHAPYR TREATED SUNFLOWER PLANTS

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Molecular characterization of resistance to AHAS-inhibiting herbicides in sunflower requires the study of gene expression in resistant and susceptible treated plants. Disruption of cellular division mechanisms is a well known effect of imidazolinones, so in order to study novel resistance mechanisms it is necessary to evaluate herbicide-treatment response over entire plants, avoiding leaf expansion times. The objectives of this study were: (i) to evaluate plant growth in response to herbicide (ii) to quantify AHAS enzyme activity. Two sunflower inbred lines HA425 and HA89 (resistant and susceptible respectively) were used. Seedlings of both genotypes were grown under controlled conditions of photoperiod and temperature. Herbicide treatment (imazethapyr 1 μ M) was performed at day 8. Total leaf area, leaf length, and hue angle were evaluated after 0, 1, 2, 3 and 4 days of treatment (dot) using *Tomato Analyzer 3.0* software. AHAS *in vitro* activity was quantified by Westerfeld colorimetric method. Non significant leaf growth occurred before 1 dot. To determine whether AHAS was inhibited in this period, AHAS *in vitro* activity was quantified by Westerfeld colorimetric method at 12, 18 and 24 h of treatment (hot). A significant AHAS activity reduction was observed in susceptible treated plants at 12, 18 and 24 hpt, suggesting that the herbicide reached its target site. It is concluded that the first 24 hot would be the ideal time interval to perform gene expression studies in leaves because at this time leaf-expansion genes would not be activated.

A64

EXPANSIN PROTEIN INVOLVED IN SOYBEAN GERMINATION AND ITS CONTROL BY ABA.

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Seed germination begins with water uptake and ends with radicle protrusion through seed coat. This process is driven by the elongation of a definite region from embryo axis known as Elongation Zone (EZ) but not involve cellular division. In

order to this expansion occurs, changes controlled metabolically in the cell wall plasticity must produce to allow this irreversible increase of volume. A group of non hydrolytic proteins known generically as Expansins were postulated as primary agents for this role. On the other hand, the abscisic acid (ABA) is a known inhibitor of germination and its action would operate on initial stages, preventing the relaxation of the cell wall and the subsequent uptake of water. The object of this work was to evaluate the role of Expansins on soybean germination and its possible regulation by ABA. Embryonic axes of soybean (*Glycine max* L. Merr) at physiological maturity (45 day after anthesis) were incubated in distilled water (DA) or 50 μ M ABA. Specific primers were designed by BLAST homology with orthologous genes from *Arabidopsis thaliana* and *Medicago truncatula*. cDNA from EZ of germinated axes was used as template in PCR reaction. The amplified product had 870 bp and showed high homology of sequence with Expansin1 of soybean. The abundance of mRNA for that product was studied from ZE of embryonic axes incubated at 0, 6 and 12 h in DA or ABA by qRT-PCR, using the internal reference gene Glyma05g37470. Expression studies indicated that this transcript is over-expressed during incubation in DA, instead decreases in 50 μ M ABA. These results would confirm the involvement of Expansin 1 on soybean seed germination, its induction during incubation in DA and the inhibitory effect of ABA on germination by repressing its expression.

A65
ESTIMATION OF MOLECULAR VARIATION COMPONENTS IN BANANA (*Musa* spp) BY AMOVA

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The AMOVA (Analysis of Molecular Variance) allows showing the effect of certain classification factors on variation of a molecular attribute in a population. Components of molecular variation were estimated in 56 banana clones collected in northern Argentina and characterized by AFLP markers. Three measuring criteria of population subdivision were considered in a hierarchical (or classified) structure. Ploidy was the first criterion (autotriploids clones -AAA- vs. allotriploids -AAB-). The use of clones as second criterion differentiated those cultivated for fresh consumption from those cultivated for cooking and from the varieties used as testers (introduced 50 years ago and which have originated the current populations of northern Argentina). The origin of subpopulations as third criterion differed according to the geographical location where the clones were obtained. All sources of variation were significant. The ploidy and use explained most of the variation. The few differences between origins and high variation within populations suggest that the sampled populations are heterogeneous (polyclonal) but similar, possibly due to the exchange of plant material between producers and natural or intuitive artificial selection.

A66
QTLs DETECTION FOR MORPHOLOGICAL TRAITS AND POST HARVEST LIFE IN TOMATO FRUITS (*Solanum lycopersicum*)

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Morphological traits and post-harvest life are economical important attributes. The AFLPs (Amplified Fragments Length Polymorphism) are dominant, multiloci and allow a wide genome cover. The goal of this research was to detect QTLs (quantitative traits loci) in a F₂ segregant population obtained from the second cycle hybrid (ToUNR1xToUNR5) using 6 AFLP primer combinations and seven quantitative traits: post-harvest life (PHL), diameter (D), weight (W), height (H), shape (S), color (L) and firmness (F). The normality of each trait was evaluated using the Shapiro-Wilk's test. The χ^2 -test was used to verify the Mendelian segregation of each polymorphic band and the segregation of each pair of fragments to prove the hypothesis of independence. Associations among the polymorphic fragments showing the expected segregation and the phenotypic traits were detected by ANOVA single point analysis. Only D and F were normally distributed. H, W and PHL were transformed with the Log₁₀ function. S and L were analyzed using the Kruskal-Wallis method.

Amplifications revealed a total of 137 fragments, of which 74 (54%) were polymorphic and 28 of them (38%) had a Mendelian segregation. Fifteen QTLs were detected: three fragments associated with D, H, S and W; two for PHL and one for L. No QTLs were detected for F. There were pleiotropic effects for four fragments (148II, 102HH, 303II, 155II). No epistasis effects were found for the eleven pairs of independent fragments. The AFLP markers allowed to detect QTLs associated to several economical important traits and they let find pleiotropic effects.

Facultad de Ciencias Veterinarias

A67

USE OF POLIMERASE CHAIN REACTION TO DETECT HEMOTROPHIC MYCOPLASMA IN DOG BLOOD. FIRST REPORT IN ARGENTINA

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Hemotrophic mycoplasma (HM) can infect different mammalian red blood cells. Infected animals suffer anemia when some conditions (most of them without identification) are present. These epierythrocyte bacteria can be detected by May Grünwald-Giemsa stained blood smears, but this method have a lot of false positive results. Polimerase chain reaction (PCR) can detect HM with high sensitivity and specificity. The aim was to use PCR to detect HM in blood of dogs without signs of anemia. Seventy one dogs that live in homes (6), kennels (25), and breeds (40) were studied. A sample of blood with EDTA was collected from each dog. DNA was extracted using a rapid extraction DNA kit (DNeasy Blood & Tissue Kit, Qiagen®). A 700 bp region of RNAr16S gene was used to detect HM (HemMycop16S-322s and HemMycop16S-938as primers). Hemotrophic mycoplasma DNA was detected in 51 samples; 76% of the kennel's dogs (19/25), 77% of the breed's dogs (31/40) and 33% of the home's dogs (2/6) were infected. Animals with positive PCR result were considered HM infected. The percentage of infected animals was lower ($p < 0,05$) in home's dogs than those of kennels and breeds, probably because a lot of animals living together help HM transmission. As future work, we will extend the study to identify if this is the case. To the best of our knowledge, this is the first HM study in dog blood using PCR in Argentina.

A68

IN SACCO RUMINAL DEGRADATION OF FODDER GROWN IN THE AREA OF CASILDA, SANTA FE, ARGENTINA

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The aim of this study was to describe the kinetics of *in sacco* ruminal degradation of dry matter (RDDM) of fodder from vegetable species grown in the area of Casilda, Santa Fe, Argentina. Fodder samples used were **stubble after harvest**: wheat straw, maize, sorghum and soybean stubble; **fodder hays**: foxtail millet, festuca and alfalfa rolls (1) and alfalfa bales (2, 3, 4, 5, 6 and 7) and **prairies**: oats, barley, sweet-clover, white-clover and alfalfa dried at 60°C, ground and sieved through a 2mm sieve. Samples were incubated in ASTM 230 nylon bags (pore size: 62 micron) with 3g DM (17mgMS/cm²) each for 0, 2, 4, 8, 12, 18, 24 and 48 and 72 and 96 hours were added for harvest stubble, in the rumen of two sheep with ruminal canulae fed alfalfa hay during three periods. Data observed in the RDDM were adjusted to the exponential model: $RDDM = a + b(1 - e^{-ct})$; where **a**: soluble fraction or fast degradable, **b**: slowly degradable fraction, **c**: degradation rate and **a + b**: potential degradability. The soluble fraction of fodder was the most variable with values between 3.6 and 39.8 % for wheat straw and oats pasture. The values of the slowly degradable fraction varied between 29.9 and 93.1 % for soybean and wheat straw stubble respectively. Degradation rate of fraction was also very variable, being 0.01 %/h for wheat straw and 0.128 %/h for the 6 alfalfa bale. Fifty-nine per cent of the samples showed potentially degradable fractions with values higher than 70%. The ruminal environment showed mean values of pH, ORP (mV), N-NH₄⁺ (mg/dl) and C-Oxidizable (g/dl) of 6.86 ± 0.073 ; -288.23 ± 4.93 ; 16.13 ± 0.82 and 1.44 ± 0.054 ; respectively. The ruminal environment showed optimal characteristics for the development of microbial population activity. The studied

fodder showed a high variability in the soluble fraction as well as in the degradation rate and a large proportion of the samples showed high potentially degradable fraction of dry matter.

A69

IVERMECTIN, FAILURE OF PERFORMANCE IN THE TREATMENT OF BOVINE VERMINOUS GASTROENTERITIS

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Verminous gastroenteritis is caused by nematodes of the genera *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Nematodirus*, *Oesophagostomum*. Deworming treatment of undiagnosed parasite infections in underestimated-weight animals without veterinarian intervention is a common practice among producers. These treatments have favored the emergence of drug resistant parasites. This work aimed to determine the presence of ivermectin (IVM)-resistant parasite strains. A fecal egg count reduction test (FECRT), with 30 animals from a herd of 100 steers, weighing 250 kg, was carried out. A modified Mc Master technique (1949) with the formula suggested by McKenna (2006) was used for the FECRT, while for cultivation and L3 recovery, the Gordon and Whitlock (1939) technique was employed. L3 identification was performed by using Niec identification keys (1968). Three groups were randomly formed (n=10): Group A (0.200 mg/kg IVM 1%); Group B (Ricobendazol (RBZ) 15%; 7.5 mg/kg) and Group C (untreated control). According to WAAVP guidelines, resistance is determined when FECRT values are below 95%. Reduction percentages observed in the present work were: Group B (RBZ) 97.2%, Group A (IVM) no reduction. L3 genera present were (in decreasing order): *Cooperia*, *Haemonchus contortus* (sheep parasite) and *Strongyloides*. We conclude that, in the IVM-treated group, FECRT showed a lack of efficacy, while RBZ-treatment effectiveness was adequate. It was confirmed that sheep parasite species also develops in cattle.

A70

EVALUATION OF THE AGGLUTINATING ANTIBODIES KINETICS FOLLOWING VACCINATION AGAINST LEPTOSPIROSIS IN ADULT DOGS. PRELIMINARY RESULTS

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The referential technique for leptospirosis is the microscopic agglutination test (MAT), which detects IgM and IgG agglutinating antibodies. There are variables that can influence the interpretation of results such as titers of residual antibodies or the recent application of an immunogen against *Leptospira spp.* The aim of this work was to analyze the kinetics of agglutinins in dogs following vaccination to evaluate its possible incidence on the serological diagnosis. Thirty-one female and male adult dogs from a refuge in Carcaraña, Santa Fe, Argentina, were studied. Blood serum was collected before vaccination with a dose of inactivated bivalent Nobivac® Lepto and 15 and 30 days after. Sera were analyzed with MAT. Besides *Icterohaemorrhagiae* and *Canicola* included in the vaccine, *L. interrogans* serovars: Pomona, Grippotyphosa, Pyogenes and Castellonis were also studied. Sera collected before vaccination were negative; while of those collected 15 days after vaccination, 22 (70.96%) were positive with titers up to 1:400 to *Icterohaemorrhagiae*, *Canicola* and *Castellonis*. At 30 days, 16 positive sera (51.61%) were found, 4 had a titer to *Icterohaemorrhagiae* and 12 to *Canicola*. These results led to the observation that some dogs, after being vaccinated with immunogens against *Leptospira*, develop specific agglutinine titers that are captured by the MAT and may mislead their interpretation in the serological diagnosis of leptospirosis.

A71

APPLICATION COSTS OF POLYPHENOLS TO CONTROL FLIES IN LAYING HEN HOUSES

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This research was aimed to show that the combination of Integrated Pest Management (IPM) with another complimentary, such as Llovera's method, was able to control fly population; so, it is used in small and medium-sized farms due to the fact that its implementation requires certain care and it is difficult to implement in larger units. The aim was to add the use of polyphenols of red *quebracho* in the diet of laying hens as an alternative in the IPM to evaluate the impact on fly population in laying hen farms and to determine if it is economically viable. Two hen-houses with 5000 hens each, owned by a laying hen establishment from Pujato (Santa Fe province) were used. The preliminary positive results in regard to the control of fly larvae allowed to move forward in the control of direct costs for the application of technical and to compare them with the traditional alternatives such as chemical control and cultural practices. Values that are described are expressed in August 2014 current pesos. Two hundred kilos of polyphenols per year were used at a cost of \$45/kg = \$9000/year, it must be added a complimentary insecticide treatment during summer season consisting of 4 L DDVP at \$207 = \$828. The treatment total cost equals to \$9828 per year. The alternative method with which the comparison was made implies the use of Cyromazine at 500 g/feed ton during 3 months at \$ 17 = \$765; 4 liters of DDVP at \$207/L = \$828; 4000 kg of lime at \$3/kg = \$12000, and labor to remove *guano* and lime application was of 365 hours at \$50 = \$18250. The annual total cost of \$31843 implies a difference of \$22015 between treatments for this farm size. Results obtained by polyphenol application on fly control and cost difference make up a technical and economically viable alternative, contributing in this way to achieve a greater efficiency in IMP, and positively impacting on environmental, social and economic sustainability.

A72

REPRODUCTIVE PERFORMANCE AND CHARACTERISTICS OF THE BIRTH IN THE CHIHUAHUA RACE

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The study of 50 births of female dogs of breed Chihuahua, with the objective of determining, average of puppies by litter, percentage of dystocia on all deliveries, percentage of males and females born and causes of dystocia, as well as incidence of congenital pathologies clinically detectable. The average age in dogs with normal births was 2.7 years with an average of 1.8 births per dog, while in females with difficult births values were 1.8 and 2.9 respectively. Out of a total of 50 births 23 normal deliveries and 27 dystocic developed, establishing that dystocia due to fetal was the largest cause of dystocia with 50%, followed by dystocia by atony primary 45%, and 5% secondary atony. All of puppies born was 132 puppies, 2.64 puppies by Mia Farrow, if you perform the comparison between births of delivery normal 2.87 and dystocic 2.44 puppies per litter, there are no statistically significant differences between the eutocycs born in childbirth and newborns in dystocic. When it evaluates the percentage by sex 57% are male and 43% female. The assessment of the viability of the puppies born determines that those born by normal delivery occurred in 28% of perinatal deaths, while in those born by dystocia 16.5%. A puppy born all manifested with hydrocephalus and one with intersex. Therefore, it is concluded that race is a race with potential difficulty in childbirth, so it must be followed neatly during gestation, being that there is a lower percentage of neonatal death in births by caesarean section than in normal childbirth.

A73

EFFECT OF THE PHOTOPERIOD ON THE TESTICULAR AND EPIDIDYMAL MORPHOMETRY IN CHINCHILLA (*Chinchilla lanigera*).

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Although the assessment of testicular volume and sperm functional activity of captive Chinchilla (*Chinchilla lanigera*) subject to natural photoperiod, suggests that they are fit to breed all year round, there is no information about controlled photoperiod. Objective: to compare some morphometrics measurement of testis and epididymis as indicators of reproductive performance of male, under two regimes of artificial lighting. Adult and clinically healthy males, housed in individual cages, subjected to a controlled environmental temperature (16 - 24 °C) and humidity (<70%), were used. A photoperiodic control consisted in a natural light replacement by electric light (36W fluorescent tubes, incident luminous intensity of 25-250 lux and color temperature more than 5000K), implemented two phases of photoperiodic promotion (10-13 hs of light/day with a daily increase of 1:24 min), five months each (1/12-30/4 and 1/6-31/10) with a period of one month (May-Nov), with 10 hs of light. The animals were divided into two groups. At the end of April (Group I) with 13:30 hours of light/day and at the end of June (Group II) with 10:30 hours of light per day. A total right orchiectomy was performed in five animals/group. The following variables were determined: body weight (PA), testicular volume (VT cm³), absolute testicular weight (PTA g), body-somatic index [IOS = (PTA/PA) x 100], absolute weight (PEA) and relative epididymal weight [PER = (PEA/PTA) x 100]. The effect of day length at the time of sample collection (U test of Mann-Whitney) was statistically significant (P<0.05) only for PEA. The values observed suggest that under specified management conditions, males are competent to reproduce under both regimes of illumination. This information confirms and complements the available for specimen of captive chinchilla under a natural photoperiod regime.

A74

LOW VOLTAGE ELECTRIC CURRENT POST-MORTEM EFFECT IN *Trichinella spiralis* LARVAE IN C57 BLACK MICE DIAFRAGMATIC MUSCLE

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Trichinellosis is a parasitic disease caused by the genus *Trichinella*. The aim of this study was to determine the effect of electric current on *Trichinella spiralis* larvae encysted in diaphragmatic muscle. 21 mice (strain C57 Black) infected in 3 groups of 7 animals each were used, with the following treatments: T1) 5 V, 20 minutes of exposure, T2) 25 V, 20 minutes of exposure, and T3) control group (no treatment). Electric current was passed over the diaphragmatic muscle with an electrophoresis generator. Muscles were artificially digested for 30 minutes and larvae were classified as live or dead with the microscope. The number of dead larvae over the total larvae was calculated. The values obtained were: T1 = 0.38 ± 0.09, T2 = 0.70 ± 0.02, CG = 0.13 ± 0.02. The analysis was performed with a Tukey Multiple Comparison statistical test. The data analysis determined no statistically significant difference between T1 and Control Group (p <0.05), but a statistical significant difference between (T2) and (T1,CG), showing a about 70% mortality rate. These results show that the only effective treatment was T2, inactivating 70% encysted larvae in the mice diaphragms

A75

USE OF POLIMERASE CHAIN REACTION TO DETECT HEMOTROPHIC MYCOPLASMA IN DOG FLEAS. FIRST REPORT IN ARGENTINA.

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Fleas transmit different diseases, including hemotrophic mycoplasma (HM) between dogs. HM can infect erythrocytes of different mammals and produce mild to serious anemia. Blood is the most important way of transmission but transmission via spittle is also possible in cats. *Ctenocephalides felis* produce 96% of dog flea infestations, but *Ctenocephalides canis* predominate in some populations. It is unknown if *C. felis* and *C. canis* have the same importance in HM transmission. The aim of this work was the use of the polymerase chain reaction (PCR) to detect HM in *C. felis* and *C. canis* collected from dogs. Fleas were obtained from 65 kennel's and breed's dogs combing them during 10 minutes. Each flea was observed by stereoscopic magnifier for species identification. Flea pools (each one has 10 a 15 fleas) of *C. felis* and *C. canis* were formed: 9 *C. felis* pools, 5 *C. canis* pools and 1 *C. felis* and *C. canis* mixed pool. DNA was extracted from pools using a rapid extraction DNA kit (DNeasy Blood & Tissue Kit, Qiagen®). A 700 bp region of RNAr16S gene was used to detect HM (HemMycop16S-322s and HemMycop16S-938as primers). All pools (except one of *C. felis*) had a positive PCR result. As future work, we will extend the study to investigate if this is a statistically significant difference. To the best of our knowledge, this is the first HM investigation made in fleas collected from dogs in Argentina.

Facultad de Odontología y otras Unidades Académicas

A76

DIETETIC HABIT, EDUCATIONAL LEVEL AND DMF INDEX: IN ISLANDER WOMEN OF COAST AND NEIGHBORING ISLANDS.

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The islander women living far from dental centers lapsing pregnancy and lactation, with mobilization of their calcium and phosphate of bones and teeth for children's development and quality of their milk, receive these components if incorporated milk to your diet. Objective: to study milk consumption habits, educational level and oral status with DMF index. A standardized anamnesis, from 15-45 years, living in the "coast", concurrent urban dental care centers and the "Backwater Valerio", inhabiting islands "Charigüé" and "Wintering" with school, both without dentist were applied. Pregnancies, maternal adherence to breastfeeding, milk consumption, formal study years separated in GA up to 6 years and GB up to 12 years or more. DMF index was obtained in each group by level of study. Fisher analysis. Total interviewed: 40% were women "coast", mean age 30 years; was raised from one to four children, nursed 75%; "Charigüé" 47% women; average age 32; was raised from one to eight children; nursed 100%; in "Wintering", 45% women, mean 31 years, with one to four children; nursed 100% (p=0.5); milk consumption: in the "coast" never consumed 25%, sometimes and everyday 37.5% respectively; "Charigüé" sometimes 57% and daily 43%; "Wintering" sometimes 40% and daily 60% (p= 0.43); "coast" education -GA, 50%, DMF index 5.25; -GB 50%, DMF 11.75; "Charigüé" -GA 29% , DMF 22; -GB 71, DMF 6.6; "Wintering" -GA 20%, DMF 8; -GB 80%, DMF 6 (p= 0.029). In the "coast" was less consumption of milk, pregnancy and nursed; more availability of schools but the education did nothing to diminish the value of the index. On islands with schools by not dental centers, women consumed more milk, crossed pregnancies and nursed and a higher educational level minor index value.

A77

ODONTOBLASTIC CHANGES ON DENTAL PULP FROM GERMS AND MOLAR WITH TWO DIETS.

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Odontoblasts, dental pulp synthesize predentine primary, secondary and tertiary by caries or external stimuli. Objective: to stimulate the synthesis with a sugary diet by evaluated changes in odontoblasts in touch. Eighteen robust rats line "I" and fifteen small line "e" were subjected to usual diet nine and six (G1) and cariogenic, water with 10% sucrose, nine in each line (G2), sacrificed in three weeks, excised jaws, demineralized, removed collagen and stained with H&E. With MO 1000 X, predentine type, perimeter thickness classifying in thin (1-2 rows of odontoblasts) and thick (3 and more rows), spaces between them in few and many; size in small and large and its connection with similar in the pulp. Fisher test. In 17 dental pulps germs, 100% primary both lines and groups are found; line "I" thin perimeter both groups; on line "e", thin in G1 and thick on G2; at regular intervals some few and small spaces united a similar in dental pulp. On line "I" 14 days: 35 molar, both groups, there was secondary (p=0.007) and tertiary (p=0.03); in the first few and small spaces between them, 100% united a pulp in G1 and 28% in G2. In tertiary was 100% many and small spaces all united in G1, at 15% in G2, 54% small, united 46%. 21 days: 32 pulps with tertiary (p=0.009); few 50%, 60% small, 90% united in G1, 33% few, 78% small, 67% united in G2. 28 days: in 38 molars secondary; 57% few, 93% small, 64% united in G1, few 36%, small 100% and 78% united in G2. Line "e" 28 days: in 33, secondary (p=0.004), 57% few, 93% small, 64% united in G1, 36% few, 100% small, united 78% in G2. In germ line "I" G1, the primary with thin perimeter; on line "e" G2 this was higher by effect diet and robustless; spaces small united to pulp both lines and groups. In molars, both lines, predentine was accompanied by thin G1 and thick G2 perimeter. In different proportions small spaces united to the pulp. Spaces between odontoblasts were affected in size, number and union a pulp.

A78

HEIGHT APEX, INDEX DMF AND CONSUMPTION OF FOOD NIGHT BRUSHED POST IN THREE SUBPOPULATIONS OF ROSARIO CITY.

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Caries is an infectious disease multifactorial that depends on the interactions between the hard tissue of the tooth, the metabolic products of the diet and the biochemical in the bacterial plate when it is not removed, for the brushed one. It is related to the dental anatomy, consume of sugars and habits of oral hygiene. The dental anatomy with sharp apexes and deep ruts they are ecological niches for the accumulation of bacterial plate. A way of quantifying the dynamics of the caries is the index DMF that he considers to the tooth as a unit. The consumption of sugary food brushed night post, favors the production of acids for the mouth flora. The aim of the present study was to relate the index DMF, height apex and consume of sugars post brushed nocturne in assistants to Centers of Dental Attention in different areas of our city. With a standardized anamnesis there were investigated information of age, sex, index DMF, height apex classified in sharp and low, consume of adhesive food brushed night post in 15-45-year-old persons of Rosario's city. One worked with following institutions: 1) private attention; 2) public: a) of the provincial area; b) of the municipal area. Of the total, 333 persons, 22 % was particular, 26% municipal and 52% to the provincial hospital (p<0,001); average age 32 years in the private one, 24 in municipal and 26 in the provincial area; 34 % of males and 66 % of women (p<0,001). Average DMF private group 25 ±4,26, municipal 23 ±4,34 and provincial 25 ± 4,10. Sharp apexes 11 % and lower 82 % in the private service; 13 % and 80 % in the municipal and 26 % and 69 % in the provincial area respectively (p=0,095). In the private one, the food consumption post brushed nocturne was 43 %; in municipal of 65% and in the provincial area of 52 % (p=0,018). The average level of the Index DMF does not present significant differences between the different groups. Our population was integrated wholesale proportion of young women. There was majority of low apexes in three sub-populations, therefore this variable would not be a factor of risk for the development of caries. Difference was not situated with regard to the indexes DMF, it would be of usefulness to discriminate between decayed, lost and plugged teeth. The consumption of sugary food brushed night post, was minor in the private sector, showing it differs from behavior between services being a factor of risk for the dental health in the public services.

A79

DETERMINATION OF DIFFICULTY IN ANSWERS TO SUBJECTS OF 1ST TEST OF BIOCHEMISTRY IN THREE COHORTS OF 2nd YEAR MEDICINE STUDENTS.

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Critical reflection of teaching practice allows review of teaching strategies used. Objective: To determine the level of difficulty of replies to topics evaluated in the 1st test of Biochemistry on 3 cohorts of students, 2012, 2013 and 2014. Material and methods: Written answers to 8 questions on different subjects evaluated at 1st test of the subject in the years 2012, 2013 and 2014 were analyzed. 59 students in 2012, 62 of 2013 and 50 of 2014. Each answer has been rated with good-B-, regular-R- and wrong -M- to analyze the issue difficulty presented. To determine whether there is a statistically significant connection between the results obtained for each question in years 2012, 2013 and 2014, the Pearson's Chi-Square Test was applied with a confidence level $\alpha = 5\%$ and the Contingency Test C was applied too to observe the degree of association. Results: In the thematic 1, 3, 6, 7 and 8 correct answers improved in 2013 compared to 2012, but worsened in 2014 compared to 2013. Thematic 2 and 5 fewer correct answers in 2013 compared to 2012. But in both, was a significant increase in correct answers for 2014, surpassing the number of the years 2012 and 2013 was observed. Thematic 4 shows the correct sustained over 3 consecutive years increased responses. Conclusion: 4 out of 8 subjects evaluated had lower level of difficulty in the 1st test of 2013 but higher degree of difficulty in the 1st test of 2014. In the other half of the evaluated thematic lower degree of difficulty is observed for both 2013 and 2014.

A80

ANALYSIS OF EDUCATIONAL INTERVENTION BY AUXILIARY STUDENTS FROM BIOCHEMISTRY SUBJECT 2nd YEAR MEDICAL CAREER.

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Biochemistry is an annual course for 2nd year of medicine. Auxiliary - AA - biochemistry course students are advanced students in the career that acted as tutors in the educational intervention carried out with 2nd year students. The AA guided the process training student, because that mentoring is a complex and dynamic phenomenon with a sociocognoscitivo character. A previous exercise was done - TP - 1^o it is a similar test like would be evaluated in the official exam. Student put a note of self-assessment before to the correction of the teacher-TP1 - and another after this - TP2-. Auxiliary students conducted an educational intervention of tutorials, in which students had to sign up voluntarily, obtaining the participation of all students. In the 1st exam student put a note of self-assessment - 1^o P1 - and then the teacher assessed the exam - 1st P2. Self-assessment in the TP1 notes are greater than the TP2 notes. The notes of self-evaluation in the P1 are greater than P2 notes. Conclusion: auxiliary intervention had a positive impact on the evolution of the capacity of the student of self-assessing their knowledge.

A81

THE CHARACTERIZATION OF THE WAYS ABOUT SELF-EVALUATE OF THE STUDENTS IN THE BIOCHEMISTRY SUBJECT IN MEDICINE IN THE SELF-EVALUATION PROCESS IN 2013.

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The reflection of the learning and teaching process could be addressed through a strategy as the self-evaluation. It's used this type of evaluation to promote in the students the critical reflection about their process of learning and teaching.

Biochemistry, an annual subject of 2^o year of medicine promotes the self-evaluation of the students. About the results, was inferred that the students doesn't evaluate responsibly and was necessary continue the training in this competence. Materials and methods: 59 students did the first partial; they self-evaluate putting a note when they finished the exam that compares with the note obtained in the correction did by the teachers; in the second, 58 students; and 49 in the third. Was used the Chi-cuadrado de Pearson test; with a signification level of 5% and the contingency coefficient C to determine the association grade in case of the variables were statistically related.

The difference is statistically significant for this group studied. The matches between the notes of the self-evaluation and the obtained in the partial, in the three partials, increased while the overestimates notes decreased as they do the different partials. Is concludes thatdoing the successive exercises of self-evaluation the students reach or improve the competence of self-evaluation.

A82

TO DETERMINE THE LEVEL OF IMPORTANCE THAT THE CONTENTS OF THE SUBJECT BIOCHEMISTRY IS GIVEN TO THE HIGHER LEVEL MED-STUDENTS.

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Aim: To determine the level of importance of the developed contents in Biochemistry appraised by advanced students. Methods and materials: There's a closed survey based on seven of the developed contents in the Subject Biochemistry. It's self-administered to 70 (seventy) students in the 3rd to 6th year. Findings: The 3rd and 4th year students found as "very valuable": 10/10 students considered the involvement of the metabolism in different tissues; 9/10 enzymes, blood and urine; 8/10 protein biosynthesis, process and regulation, bimolecular metabolism and nutrition. The 5th and 6th year students found as "very valuable": 10/10 enzymes, the mechanics of signals transduction, blood, urine and nutrition; 9/10 bioenergetics – water – structure and properties, bimolecular metabolism, participation of the metabolism in different tissues, genetic abnormalities in the growing cells; 8/10 introduction to Metabolism. There is an agreement in the qualification as "very important/valuable" in all the referents to the subjects of blood, urine, nutrition, metabolism involvement in different tissues and the biomolecular metabolism. Conclusion: We can infer that the contents in the Biochemistry have a relevant importance for the students in the higher years.

A83

COLONIZATION OF *Lactobacillus salivarius* DSPV 001P IN THE GASTROINTESTINAL TRACT OF BROILERS

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Survival of probiotic bacteria through the gastrointestinal tract is crucial to exert a positive effect when administered in broilers. The aim of this study was to assess the colonization and persistence of lactic acid bacteria (LAB) of avian origin with probiotic properties in the gastrointestinal tract (GIT) of broilers for 44 days. Rifampicin resistant *L. salivarius* DSPV 001P was selected to monitor bacteria during the study. Ninety six 1 day old Cobb broilers were used in the trial. There were three replicates with 32 broilers per replicate. Experiment lasted for 44 days. The strain was administered to the diet at a dose of 1×10^{10} CFU/broiler during 9 days and 1×10^9 CFU/broiler during 7 days. On day 0, 48 h post administration and every seven days, six broilers (two per replicate) were slaughtered by cervical dislocation. To measure the GIT colonization, the number of CFU recovered from crop and caecum was determined. On day 0, there were no rifampicin resistant bacteria detected from broilers' crop and caecum. After two days of feeding, the strain was found at levels of approximately 3.73 ± 1.88 log CFU/crop and 4.97 ± 0.31 log CFU/caecum. Throughout the 16-days feeding period the level of *L. salivarius* DSPV 001P increased to 7.87 ± 0.44 log CFU/crop and 7.41 ± 0.35 log CFU/caecum. Total cell count on day 44 was 6.75 ± 1.67 log CFU/crop and 6.22 ± 2.26 log CFU/caecum. *L. salivarius* DSPV 001P is capable of persist in the GIT of broilers during a complete rearing. Probiotic strains persistence following cessation of administration is the ideal situation for producers, as the strains do not necessarily need to be used as ongoing in-feed additives.

A84

THE RELATION BETWEEN THE PASS MARK OF FIRST YEAR BASIC SUBJECTS AND THE STUDENTS' ACADEMIC RESULTS OF IN THE FIRST SECOND YEAR BIOCHEMISTRY TEST.

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Our interest is to evaluate the students' academic results in Human Anatomy and Histology, Cytology and Embriology compared to their results in the first Biochemistry assessment so as to determine if there is a close relationship between de latter to the former ones. First year students' marks were considered together with their 2014 Biochemistry marks. Total of students': 49. The Pearson Chi Cuadrado Test was applied with a 5% relevance. Contingency Test was used to see the association variables. Results: 46 students passed the final Histology, Cytology y Embriology test. 24 students passed the final Human Anatomy test. 36 students passed de first Biochemistry test. Conclusion: there is a close relation between the approval of final Human Anatomy test to the approval of the first Biochemistry test.

A85

USE OF CHEESE WHEY PERMEATE AS CRYOPROTECTANT OF AN FREEZE DRIED PROBIOTIC INOCULUM FOR CALVES

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The objective was to evaluate the use of cheese whey permeate (P) as cryoprotectant to maintain the viability of a probiotic strain during lyophilization and storage at different temperatures. The biomass of *Lactobacillus casei* DSPV318T of bovine origin, was grown in P (6% w/v) and hydrolyzate of cheese whey (7% w/v). The culture was washed with distilled water and resuspended in cryoprotectant solutions: P (6% w/v) and skim milk (6% w/v). These were compared with the negative control: distilled water. The vials were freeze dried and stored at room temperature (RT) and refrigeration. The bacterial viability was determined pre and post freeze drying and every seven days during 28 days of storage by performing decimal dilutions and plate on MRS medium. Viability of bacteria in both cryoprotectants remained above the suggested minimum level (SML) (10^6 Log (UFC/g)) for 21 days in the refrigerator. The inoculum with cryoprotectants but at RT and inoculum without cryoprotectants in both temperatures were maintained over the SML by 7 days. The P was able to protect *L. casei* in the freeze- dry process just as the cryoprotective normally used. The cheese whey permeate decreases production costs and the probiotic inoculum viability to maintain proper administration to calves.

A86

PARASITISM AND PRODUCTION OF INFECTIVE JUVENILES OF *Steinernema rorum* (OLI) (NEMATODA: STEINERNEMATIDAE) IN ADULTS AND LARVAE OF *Anticarsia gemmatalis* (INSECTA: LEPIDOPTERA) IN LABORATORY

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Anticarsia gemmatalis is a soybean pest. The entomopathogenic nematode *Steinernema rorum* (OLI), is a natural controller of the pest populations. The aims of this work were: 1) to evaluate the susceptibility of adults and larvae of *A. gemmatalis* to *S. rorum* (OLI); 2) to determine the occurrence of infective juveniles (IJs) at the end of parasitic cycle and to estimate their number; 3) to compare mortality of adults and larvae and the number of IJs. Two doses were used: 50 and 500 IJs/insect, and 15 individuals/stage/dose were considered. The percentages of mortality were: in adults, 33% and 80%, at 50

and 500 IJs; in larvae, 93% at two doses. Mean values of IJs were: in adults, 66621.53 ± 30504.18 and 58149.93 ± 20187.57 , at 50 and 500 IJs; in larvae, 20499.68 ± 18154.93 , and 25247.20 ± 7424.21 , to each doses. Significant differences in the number of IJs were detected between stages but no differences were detected between doses (ANOVA, $p \leq 0.05$). It is demonstrated that both adults and larvae stages of *A. gemmatilis* are susceptible to *S. rarum* (OLI), and they permit the nematode to reproduce and complete its cycle. Field work is needed to improve the knowledge of this promising control agent.

A87

LYOPHILIZED MACROCAPSULES TO MAINTAIN VIABILITY OF PROBIOTICS FOR CALVES.

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The viability and number of probiotic microorganisms inoculated is vital because the suggested minimum level (SML) of bacteria to produce beneficial effects is 10^6 CFU/ml. The aim of this study was to evaluate the viability of probiotics in cheese whey and sodium alginate macrocapsules stored in different temperature conditions. The strains were grown in 5 ml of cheese whey at 37°C for 24 h. The culture was mixed with an alginate solution in a proportion of 1:1. This mixture were dispersed into molds of 1 ml, placed at -20 °C, and, once frozen, submerged in a solution of CaCl₂ for 1h. Half of the capsules were suspended in 0.4% chitosan solution w/v for 40 minutes to create an outer coating. Half the capsules were stored at 5 °C and the rest at room temperature (RT). Bacterial concentration was determined before lyophilization and subsequently at 7, 14, 28, 42, 56, 70 and 84 days. The viability of the inoculum was higher ($P < 0.001$) at refrigeration temperature. The capsules stored at 5 °C maintained viability up to 6 log₁₀/g until day 42. In contrast, the capsules conserved at RT maintained viability up to SML until day 7. The chitosan coating had no significant effect ($P = 0.227$) on the viability of encapsulated strains. The capsules have a high concentration of probiotic, with a sufficiently long time expiration for administration in artificial breeding calves.

A88

PREVALENCE AND EVOLUTION OF RESISTANCE IN *Shigella* spp.

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The fecal-oral route is the main mode of transmission of *Shigella* spp. through ingestion of contaminated food or water, children of 1-4 years are the most affected. Most diarrhea is self-limiting and therefore must be handled only with adequate oral rehydration therapy. A small percentage of *Shigella* infections needs antimicrobial therapy. The aim of this work was to study the prevalence of *Shigella flexneri* and *Shigella sonnei* in stool cultures from patients at Children's Hospital Zona Norte January to July 2014 and evaluate the antimicrobial susceptibility to ampicillin, trimethoprim + sulfamethoxazole (TMS), ceftriaxone and furazolidone used in the treatment of diarrhea *Shigella* spp.. Compare data with values obtained in the year 2004. Were processed by standard techniques (CLDE agar, blood agar, MacConkey sorbitol, Salmonella Shigella agar and selenite broth) coprocultures of 313 patients between 0 and 13 years from January to July. Bacterial identification and antimicrobial susceptibility was performed by Vitek automated system. *Shigella* spp prevalence was evaluated and data were bought with those obtained in 2004. In 2014 the prevalence of *Shigella* was 11% (6% *Shigella flexneri* and 5% *Shigella sonnei*) 58% were resistant to ampicillin and 32% to trimethoprim + sulfamethoxazole. In 2004 was 9% (6% *Shigella flexneri* and 3% *Shigella sonnei*) 45% resistant to ampicillin and 24% trimethoprim + sulphamethoxazole. Increased prevalence of *Shigella* spp was observed in the analyzed period of 2014 compared to the same period in 2004, at expense of *Shigella sonnei* with a gradual increase in resistance to ampicillin and trimethoprim + sulfamethoxazole. Increasing resistance is attributed to widespread use of antibiotics to treat diarrhea justified by the presence of fever and parental pressure to shorten the period of the disease.

A89

***Staphylococcus aureus* ISOLATED FROM SKIN AND SOFT TISSUE.**

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Staphylococcus aureus (*Sa*) is a major nosocomial pathogen and of the community. The emergence of strains resistant to methicillin and resistant to other antimicrobials represents a challenge for treatment. The aims of this study were to determine the number of *Sa* isolated from patients admitted to Children's Hospital North Zone, describe the origin of the isolates according to the infectious focus and know the profile of antimicrobial resistance. Strains of *Sa* isolated from skin, soft tissue and osteoarticular material of patients hospitalized in Hospital for Children North Zone from June 2013 to July 2014 were analyzed. The identification and antimicrobial susceptibility of isolates was performed with automated equipment Vitek. In the period analyzed 50 isolates of *Sa* were obtained. The most were recovered from soft tissue 30 (60%), followed by skin lesions, 17 (34%) and 3 (6%) of osteoarticular materials. The 88% (44) were methicillin resistant and 16% (8) of these exhibited accompanying gentamicin resistance. 12% (6) of the studied strains were methicillin sensitive. Of total isolates compared to other beta-lactam antibiotics resistance to gentamicin (18%), clindamycin (2%), erythromycin (2%) and rifampicin (2%) was obtained. All strains were sensitive to vancomycin, teicoplanin, minocycline and trimethoprim + sulfamethoxazole. Methicillin-sensitive strains corresponded to skin lesions. The strains that showed resistance-associated methicillin resistance to gentamicin were all isolated from soft tissue. A high percentage of methicillin-resistant *Sa* in patients hospitalized mainly isolated soft tissue was observed. 16% of these isolates showed resistance to gentamicin companion. Low resistance to clindamycin, erythromycin and rifampin is highlighted. No resistance to vancomycin, teicoplanin, minocycline and trimethoprim + sulfamethoxazole was observed. Meet the resistance profile is a useful tool for building adequate initial empiric therapy in patients hospitalized with infections of skin and soft tissue.

A90

HYGIENIC EVALUATION BY MICROBIOLOGICAL METHODS OF FIVE URBAN AND PERI-URBAN SCHOOLS OF BALCARCE CITY.

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The aim was performed hygienic evaluation by microbiological methods in five urban and periurban schools in Balcarce city. Foods and surfaces were analyzed using swabs, performing counts of mesophilic aerobic bacteria (MAB), Total enterobacteria, total and fecal coliforms, *Escherichia coli*, *Staphylococcus* spp and *S. aureus*, molds and yeasts, sulphite-reducing *Clostridium*, *Pseudomonas* spp and *Pseudomonas aeruginosa* (AOAC). As results, MAB were the most frequent and with the higher counts pollutants. School A: 5.6×10^3 ufc /mL in the cleaning cloth, school B: 6.2×10^4 ufc /mL in hands, school C: 7.8×10^4 ufc /mL in bread board, school D: 3.4×10^4 ufc /mL in counter, school E 2.2×10^3 ufc /mL in hands. Molds and yeasts, were founded in hands of auxiliars in school B: 3.5×10^3 ufc mL. Also was analyzed a water sample from school A, and was found *Pseudomonas* spp., MAB was 2.7×10^3 ufc /mL, and food samples (rice stew and milk powder), wherein the presence of MAB was also observed. The areas most involved for all positive results were cutting boards, countertops and hands of the kitchen staff. The presence of *Staphylococcus aureus* and *E. coli*, were not observed. These results are in agreement with the detection of flaws in the implementation of Good Manufacturing Practices during the production and distribution of lunches and snacks. The analysis has been effective to determine the hygienic level in school establishments, which will make subsequent recommendations to improve hygiene in the preparation and handling of food, and strengthen the training of assistants that was developed under the project.

A91

IMPACT STUDY IN TWO PROMOTION PROGRAMS ON ORAL HEALTH *S mutans* RECOUNTS.

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Fluoride or chlorhexidine have been largely utilized in clinical interventions and less frequently in community ones. Similar programs' findings applied in other context cannot be easily extrapolated. Objective: To compare the effect on *Streptococcus mutans* (*Sm*) post fluoride or chlorhexidine application through two preventive programs. Methods: 50 children of three Rosario, Argentina schools were selected to take part in an Oral Health Promotion Program. They were randomly assigned to two treatment groups (Fluoride, n=21), and (Chlorhexidine, n=19). During four years stimulated-whole saliva samples were taken in two intervention moments, where bacteriological culture was prepared according to standard methods. The first sample was prior (basal) to the treatment, and the second one was one week later from the last application. The treatment consisted in a weekly application of 1.23% fluoride gel, or 1% chlorhexidine gel in four successive sessions. *Sm* was identified by standard method. The obtained quantity of CFU was compared in every intervention by using a nonparametric rank-based test on R-software (version 2.14.2). Results: For treatment "F" the basal end medians were: 2010: 12.000/3.000; 2011: 18.000/11.600; 2012: 18.000/12.000; 2013: 18.000/18.000, and for "C" 12.000/7.200; 6.600/4.500; 30.000/18.000; 16.000/12.000, respectively. It was calculated the ratio of eradicated bacteria in "F", and the medians in it were: 2010=0.75; 2011=0.25; 2012=0.40; 2013=0.13, and in "C": 0.60; 0.24; 0.40; 0.25. Interaction 'School shift - Treatment - Year', and 'Shift - Year' and 'Treatment - Year' have a significance level of 5%. Conclusions: The interaction between 'Shift - Treatment' factors has a significance level ($p=0.05$). The effect of treatment was not the same in all the years ($p<0.001$), registering the most elevated values at the beginning of the program. There is not significance in the difference between the treatments ($p=0.51$).

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