

Cuban Research in Current International Journals

The following selection—alphabetical by title—reflects Cuban publishing in international medical and population health journals over the last quarter on an array of topics. Links to these journal articles may be found at www.medicc.org/mediccreview.

Assessment of infection with polyomavirus-es BKV, JCV and SV40 in different groups of Cuban individuals. Martínez PA, Kourí V, Cordero G, Correa C, Soto Y, Capó V, et al. Arch Virol. 2011 Dec 2.

We investigated the frequency of BKV, JCV and SV40 reactivation in three groups of Cuban patients by multiplex nested PCR assay of 40 paraffin-embedded colorectal neoplasm tissues, 113 urine samples, and 125 plasma samples from 27 transplant recipients, and cerebrospinal fluid (CSF) from 67 HIV-1-infected individuals with central nervous system (CNS) disorders. None of these polyomaviruses were detected in colorectal neoplasms. JCV DNA was detected in 2 of 67 patients (2.9%) with CNS disorders, but neither BKV nor SV40 was identified. BKV was found in urine from 38.5% and 28.6% of adult and pediatric transplant recipients, respectively. In adult renal transplant recipients, excretion of BKV in urine was significantly associated with episodes of acute rejection ($p = 0.012$) and with excretion of HCMV in urine ($p = 0.008$). In Cuba, the polyomaviruses studied here could not be related to colorectal neoplasms, and JCV was rarely detected in CSFs of HIV-1-infected individuals, whilst BKV reactivation was found to occur frequently in organ transplant recipients.

Autonomic dysfunction in presymptomatic spinocerebellar ataxia type-2. Montes-Brown J, Machado A, Estévez M, Carricarte C, Velázquez-Pérez L. Acta Neurol Scand. 2012 Jan;125(1):24–9.

Objectives To explore and quantify possible abnormalities in the autonomic cardiovascular regulation in presymptomatic stage of type 2 spinocerebellar ataxia (PS-SCA2). **Materials & methods** Heart rate variability (HRV) for 5-min series of RR intervals was analyzed in 48 PS-SCA2. Autonomic testing included *resting recording, standing, Valsalva maneuver, and deep breathing*. The results were compared with a group of sex- and age-matched controls. **Results** Time- and frequency domain HRV indices were significantly different between PS-SCA2 and control groups. Using two standard diagnostic procedures were identified 4 (8.33%) subjects with severe and 8 (16.66%) subjects with early cardiac autonomic neuropathy in PS-SCA2. CAG index significantly correlated with age (-0.35) and HR (0.31). **Conclusions** Our results confirm the presence of cardiovascular autonomic dysfunction in PS-SCA2 subjects.

Case Series in Patients with Zoster-Associated Pain Using *Mangifera indica* L. Extract.

Garrido-Suárez B, Garrido G, Delgado R, Bosch F, Del C Rabí M. Forsch Komplementmed. 2011;18(6):345–50.

Background Neuroimmune activation has been proposed as a source of new targets for therapeutic intervention in neuropathic pain. Vimang is an aqueous extract from *Mangifera indica* L. (common mango) that is traditionally used in Cuba for its antioxidant, anti-inflammatory, analgesic, and immunomodulatory properties. In the present case report, we determine its potential effects in patients with zoster-associated pain. **Patients and Methods** 12 patients with zoster-associated pain (6 with subacute herpetic neuralgia and 6 with post-herpetic neuralgia) received a daily dose of 1,800 mg of extract (2 coated 300 mg tablets, 3 times daily before meals) together with low doses of amitriptyline (10–25 mg/day) for 120 days. In addition to the tablets, patients used Vimang cream 1.2% as a topical agent. The average daily pain score using the Likert scale, area and rate of dynamic allodynia, rate of thermal allodynia, and frequency of burning spontaneous pain were evaluated. **Results** Pain scores and sensory abnormalities decreased significantly ($p < 0.05$) with respect to baseline data from week 4. No adverse events were reported. **Conclusion** These results suggest that Vimang could be beneficial in the treatment of neuropathic pain. However, a controlled clinical trial is necessary to confirm this hypothesis.

CIGB-247: A VEGF-based therapeutic vaccine that reduces experimental and spontaneous lung metastasis of C57Bl/6 and BALB/c mouse tumors. Bequet-Romero M, Morera Y, Ayala-Ávila M, Ancizar J, Soria Y, Blanco A, et al. Vaccine. 2012 Jan 10.

CIGB-247 is a novel cancer therapeutic vaccine that uses a mutated form of human VEGF as antigen. Being metastatic disease the most dramatic factor of tumor biology affecting patient survival and cure, preclinical evaluation of the impact of CIGB-247 vaccination on experimental metastasis mouse models is highly relevant, and constitutes the focus of this work. CIGB-247 was administered in a weekly schedule known to effectively reduce primary tumor growth. The vaccine was tested in experimental and spontaneous metastasis models of colon (CT26), lung (3LL-D122) and breast (F3II) carcinomas growing in C57Bl/6 or BALB/c mice. Primary tumor growth parameters, metastatic counts, and/or animal survival were recorded. Histology and specific humoral and cellular responses to the vaccine were evaluated. As compared to control groups, CIGB-247 vaccination significantly

reduced the number and size of metastatic tumor foci in lungs after intravenous inoculation of CT26 and 3LL-D122 tumor cells. Spontaneous lung dissemination from 3LL-D122 and F3II breast tumor cells implanted in the footpad, or subcutaneously, was also reduced by immunization with CIGB-247. The vaccine elicited in both mouse strains antibodies specific for human and murine VEGF that effectively blocked the interaction of VEGF with VEGF receptor 2. Differing from other experimental reports that describe the use of VEGF for active tumor immunotherapy, CIGB-247 elicited a specific cellular response, measured both by a DTH increment and the induction of spleen cells cytotoxic to syngeneic tumor cells producing murine VEGF. In summary our results reinforce the potential of CIGB-247 vaccination to reduce both tumor growth and the number and size of tumor metastasis in lungs, the latter both after direct inoculations of cells in the blood stream, or as part of primary tumor progression in immunocompetent mice.

Costs of dengue prevention and incremental cost of dengue outbreak control in Guantanamo, Cuba. Baly A, Toledo ME, Rodríguez K, Benítez JR, Rodríguez M, Boelaert M, et al. Trop Med Int Health. 2012 Jan;17(1):123–32.

Objective To assess the economic cost of routine *Aedes aegypti* control in an at-risk environment without dengue endemicity and the incremental costs incurred during a sporadic outbreak. **Methods** The study was conducted in 2006 in the city of Guantanamo, Cuba. We took a societal perspective to calculate costs in months without dengue transmission (January–July) and during an outbreak (August–December). Data sources were bookkeeping records, direct observations and interviews. **Results** The total economic cost per inhabitant (p.i.) per month. (p.m.) increased from 2.76 USD in months without dengue transmission to 6.05 USD during an outbreak. In months without transmission, the routine *Aedes* control programme cost 1.67 USD p.i. p.m. Incremental costs during the outbreak were mainly incurred by the population and the primary/secondary level of the healthcare system, hardly by the vector control programme (1.64, 1.44 and 0.21 USD increment p.i. p.m., respectively). The total cost for managing a hospitalized suspected dengue case was 296.60 USD (62.0% direct medical, 9.0% direct non-medical and 29.0% indirect costs). In both periods, the main cost drivers for the *Aedes* control programme, the healthcare system and the community were the value of personnel and volunteer time or productivity losses. **Conclusions** Intensive efforts

to keep *A. aegypti* infestation low entail important economic costs for society. When a dengue outbreak does occur eventually, costs increase sharply. In-depth studies should assess which mix of activities and actors could maximize the effectiveness and cost-effectiveness of routine *Aedes* control and dengue prevention.

Development and validation of a bioanalytical LC-MS method for the quantification of GHRP-6 in human plasma. Gil J, Cabrales A, Reyes O, Morera V, Betancourt L, Sánchez A, et al. *J Pharm Biomed Anal.* 2012 Feb 23;60:19–25.

Growth hormone-releasing peptide 6 (GHRP-6, His-(DTrp)-Ala-Trp-(DPhe)-Lys-NH(2), MW=872.44Da) is a potent growth hormone secretagogue that exhibits a cytoprotective effect, maintaining tissue viability during acute ischemia/reperfusion episodes in different organs like small bowel, liver and kidneys. In the present work a quantitative method to analyze GHRP-6 in human plasma was developed and fully validated following FDA guidelines. The method uses an internal standard (IS) of GHRP-6 with (13)C-labeled Alanine for quantification. Sample processing includes a precipitation step with cold acetone to remove the most abundant plasma proteins, recovering the GHRP-6 peptide with a high yield. Quantification was achieved by LC-MS in positive full scan mode in a Q-ToF mass spectrometer. The sensitivity of the method was evaluated, establishing the lower limit of quantification at 5 ng/mL and a range for the calibration curve from 5 ng/mL to 50 ng/mL. A dilution integrity test was performed to analyze samples at higher concentration of GHRP-6. The validation process involved five calibration curves and the analysis of quality control samples to determine accuracy and precision. The calibration curves showed R(2) higher than 0.988. The stability of the analyte and its internal standard (IS) was demonstrated in all conditions the samples would experience in a real time analyses. This method was applied to the quantification of GHRP-6 in plasma from nine healthy volunteers participating in a phase I clinical trial.

Effect of Bixa orellana against Leishmania amazonensis. García M, Monzote L, Montalvo AM, Scull R. *Forsch Komplementmed.* 2011;18(6):351–3.

Background In the present study, an activity of Bixa orellana extract against Leishmania amazonensis was demonstrated. **Result** Experimentally infected BALB/c mice were treated with B. orellana extract which showed a significant activity against promastigote and amastigote forms of L. amazonensis. **Conclusion** This study supports the importance of natural sources as antileishmanial drugs.

Electroencephalographic abnormalities in antisocial personality disorder. Calzada-Reyes A, Alvarez-Amador A, Galán-García

L, Valdés-Sosa M. *J Forensic Leg Med.* 2012 Jan;19(1):29–34.

The presence of brain dysfunction in violent offenders has been frequently examined with inconsistent results. The aim of the study was to assess the EEG of 84 violent offenders by visual inspection and frequency-domain quantitative analysis in 84 violent prisoners. Low-resolution electromagnetic tomography (LORETA) was also employed for theta band of the EEG spectra. Antisocial personality disorder (ASPD) was present in 50 of the offenders and it was absent in the remaining 34. The prevalence of EEG abnormalities, by visual inspection, was similar for both the ASPD group (82%) and non-ASPD group (79%). The brain topography of these anomalies also did not differ between groups, in contrast to results of the EEG quantitative analysis (QEEG) and LORETA that showed remarkable regional differences between both groups. QEEG analysis showed a pattern of excess of theta-delta activities and decrease of alpha band on the right fronto-temporal and left temporo-parietal regions in the ASPD group. LORETA signified an increase of theta activity (5.08 Hz) in ASPD group relative to non-ASPD group within left temporal and parietal regions. Findings indicate that QEEG analysis and techniques of source localization may reveal differences in brain electrical activity among offenders with ASPD, which was not obvious to visual inspection.

Epidemiological studies on dengue virus type 3 in Playa municipality, Havana, Cuba, 2001–2002. Guzmán MG, Alvarez A, Vázquez S, Alvarez M, Rosario D, Peláez O, et al. *Int J Infect Dis.* 2012 Jan 23.

Objectives Recognizing the uniqueness of secondary dengue virus (DENV-1/3) dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) cases at an interval of 24 years, we sought to estimate DENV infections as well as the ratios between mild disease and DHF/DSS by DENV infection sequence in Playa District (Havana, Cuba) during the 2001–2002 outbreak of dengue virus type 3 (DENV-3). **Methods** A retrospective seroepidemiological study was conducted in 2003 in Playa District. Blood samples were collected from a 1% random sample of residents and were studied for the prevalence of dengue neutralizing antibodies. **Results** DENV-3 was found to have infected 7.2% (95% confidence interval (95% CI) 6.0–8.4%) of susceptible individuals (the entire cohort), the majority of whom experienced silent infections. Virtually every individual who had a secondary infection in the sequence DENV-1 then DENV-3 became ill, with a ratio of severe to mild cases of 1:35 (95% CI 1:67–1:23). Secondary infections in the sequence DENV-2/3 were less pathogenic than DENV-1/3. Mild disease accompanying secondary DENV2/3 occurred at a ratio of 1:4.49 infections (95% CI 1:5.77–1:3.42) secondary infections. **Conclusions** The results obtained highlight the role of the infecting serotype and also the sequence of the viral infection in the clinical outcome of a dengue infection.

Lentiviral vector followed by protein immunisation breaks tolerance against the self-antigen Her1 and results in lung cancer immunotherapy. Alpizar YA, Karwacz K, Arce F, Rivera AY, Fernández LE, Collins MK, et al. *J Gene Med.* 2012 Jan 19.

Background Lung cancer remains a leading cause of cancer mortality, so our aim was to develop a therapeutic vaccine protocol. **Methods** We constructed a lentiviral vector (LV) expressing the extracellular domain (ECD) of murine Her1, an antigen associated with poor prognosis in lung cancer. **Results** A single LV injection, followed by two Her1 protein boosts, was effective in reducing the metastatic burden of Lewis lung carcinoma in mice. The Her1 LV immunisation generated CD8+ T cells which recognised Her1 ECD presented by dendritic cells and which homed to Her1 expressing tumours. Protein boosting further increased the CD8+ T cell response and generated anti-Her1 antibodies; in the antibody response Her1 LV priming increased Th1-dependent IgG2c production. **Conclusions** The ability of this vaccine protocol to break both T cell and B cell tolerance to a self-antigen likely explains its effectiveness.

Mebendazole compared with secnidazole in the treatment of adult giardiasis: a randomised, no-inferiority, open clinical trial. Almirall P, Escobedo AA, Ayala I, Alfonso M, Salazar Y, Cañete R, et al. *J Parasitol Res.* 2011;2011:636857.

To compare the efficacy and safety of mebendazole (MBZ) and secnidazole (SNZ) in the treatment of giardiasis in adult patients, a single-centre, parallel group, open-label, randomized non-inferiority trial was carried out. One-hundred and twenty-six participants who had symptomatic Giardia mono-infection took part in the study. Direct wet mount and/or Ritchie concentration techniques and physical examinations were conducted at the time of enrolment and at the follow-up visit. The primary outcome measure was parasitological cure, performed at 3, 5, 10 days post-treatment. Negative faecal specimens for Giardia were ensured by the same parasitological techniques. At follow up (day 10) the parasitological cure rate for the per protocol populations was 88.7% (55/62) for MBZ and 91.8% (56/61) for SNZ. For the intention to treat populations the cure rate at the end of treatment was 85.9% (55/64) for MBZ and 90.3% (56/62) for SNZ. Both analyzes showed there was not significant statistical difference between MBZ and SNZ treatment efficacy. Both drugs were well tolerated, only mild, transient and self-limited side effects were reported and did not require discontinuation of treatment. A 3-day course of mebendazole seems to be as efficacious and safe for treatment of giardiasis as a single dose of secnidazole in adults.

Ozone oxidative post-conditioning reduces oxidative protein damage in patients with disc hernia. Fernández OS, Pantoja M, Soto

MT, Insua MG, Viebhan-Hánsler R, et al. *Neurol Res.* 2012 Jan;34(1):59–67.

Introduction/objectives Although inflammation in disc hernia (DH) has been recognized and it is a well-known process mediated by loss of the cellular redox balance, only a few studies about the impact of chronic oxidative stress on this neurological disorder have been made. Ozone therapy has been widely used with clinical efficacy in DH. This work aimed at characterizing the systemic redox status of patients with low back pain and neck pain as well as studying if ozone oxidative post-conditioning modified the pathological oxidative stress and protected against oxidative protein damage and if there is any relationship between oxidative changes and pain in both DH. **Methods** Redox status of 33 patients with diagnosis of DH by computerized axial tomography, nuclear magnetic resonance, and clinical evaluations was studied. Ozone was administered by paravertebral way. After ozone treatment, plasmatic levels of antioxidant/pro-oxidant markers, pain, and life quality disability parameters were evaluated. **Results** One hundred percent of patients showed a severe oxidative stress. Major changes in superoxide dismutase activity, total hydroperoxides, advanced oxidation protein products, fructolysine content, and malondialdehyde were observed. After ozone oxidative post-conditioning, there was a re-establishment of patients' cellular redox balance as well as a decrease in pain in both DH. A relationship between indicators of oxidative protein damage and pain was demonstrated. **Conclusions** Ozone therapy protected against oxidation of proteins and reduced the pain. Relationship between markers of oxidative protein damage, disability parameters, and pain suggests the role of oxidative stress in the pathological processes involved in DH.

Prevalence of antibodies to *Borrelia burgdorferi* sensu stricto in humans from a Cuban village. Rodríguez I, Fernández C, Sánchez L, Martínez B, Siegrist HH, Lienhard R. *Braz J Infect Dis.* 2012 Feb;16(1):82–5.

Lyme disease has not been officially reported in Cuba. However, clinical cases have been serologically reported. Seroprevalence survey of *Borrelia burgdorferi* sensu stricto antibodies in humans in the country has not been conducted. **Objective** To estimate the prevalence of borrelial antibodies in inhabitants of a village with historically high level of tick infestation. **Methods** Serum specimens from 247 persons randomly selected from the population of the village were examined by IgG Western blot using B31 strain for estimating the prevalence of antibodies profile. **Results** A seroprevalence value interval (95% CI) of 0.6%-7.2% was estimated for the studied population. The prevalent borrelial protein bands on immunoblots were 41, 72, 90/93, 34, 47, 60, 58, 56, 65/66 and 31 kDa in a decreasing order of significance. **Conclusions** These results support the previous serological findings, suggesting the presence of this borreliosis in Cuba.

Testing the Effectiveness of Community-Based Dengue Vector Control Interventions Using Semiparametric Mixed Models. Sánchez L, Maringwa J, Shkedy Z, Castro M, Carbonell N, Stuyft PV. *Vector Borne Zoonotic Dis.* 2012 Jan 12.

Objective To evaluate the results obtained through intersectoral coordination and community empowerment in one study carried out during six years in Playa Municipality, Cuba. **Methods** A longitudinal assessment comparing one intervention and one control area was conducted. The intervention encompasses two main stages separated by two dengue outbreaks. The first stage, focused on strengthening intersectoral coordination, was initiated in January 2000. In August 2003, a complementary strategy, focused on community empowerment and was initiated in half of the intervention area. In the control area, routine dengue control activities continued without additional input. We used entomologic surveillance data from January 1999 to December 2005 to assess the effectiveness. We computed the Breteau index (BI) per health area and the 95% confidence interval for the difference between the BIs at each time point. A semiparametric mixed model to capture the evolution in time of *Aedes aegypti* larval densities was fitted. **Results** The BI in the control area showed the lowest value before starting the intervention. This was reversed one year after launching intersectoral activities for dengue control in the intervention area. In spite of spraying actions in all areas, the differences in BI between intervention and control areas remain significant until December 2002. Although for the next two years no differences were observed, they become significant again in December 2004, which corresponds with the implementation of the complementary community-based vector control strategy in the intervention area. **Conclusions** The model fitted identified monotonous trends over time and reversal trends at particular moments. The confidence bands indicate sections with significant differences between areas. Our data increase the evidence that the intersectoral coordination and community empowerment strategy for *A. aegypti* control is effective.

Trends in digestive cancer mortality, Cuba 1987-2008. Abreu MR, Vilar E, Arús ER, Mejía JM, Martínez Y, Yassells AA. *Eur J Public Health.* 2012 Feb 7. [Epub ahead of print]

Background Gastrointestinal malignancies are among the most common cancers suffered by Cubans. The purpose of our study is to analyse the evolution of digestive cancer mortality in Cuba. **Methods** Mortality data for this study were obtained from the National Medical Records and Health Statistic Bureau. Trends (1987-2008) in age-standardized cancer mortality were described using joinpoint regression. **Results** In the data set of digestive cancer mortality, in the period 1987-2008, colorectal/anal cancer was the most frequent cause of cancer mortality in males and females. In men,

a rise in mortality was observed for cancer of the oesophagus between 2001 and 2008, and pancreatic cancer showed a slight mortality rise for the period 1987-2008. In women, colorectal/anal cancer increased from 1989 to 2001. A mortality increase was observed for oesophageal cancer in the period 2005-08. The result of the joinpoint analysis for the age group of 35-64 years was consistent with those for overall mortality. **Conclusions** The trend in mortality from digestive cancer in Cuba shows differences depending on sex, age and type of tumour. The Cuban health system has seen improvements in diagnostic systems, which has contributed even better diagnoses of digestive diseases.

Understanding rhodopsin mutations linked to the retinitis pigmentosa disease: a QM/MM and DFT/MRCI study. Hernández EW, Sánchez E, Crespo R, Montero AL, Montero LA, Thiel W. *Phys Chem B.* 2012 Jan 26;116(3):1060–76.

Retinitis pigmentosa (RP) is a pathological condition associated with blindness due to progressive retinal degeneration. RP-linked mutations lead to changes at the retinal binding pocket and in the absorption spectra. Here, we evaluate the geometries, electronic effects, and vertical excitation energies in the dark state of mutated human rhodopsins carrying the abnormal substitutions M207R or S186W at the retinal binding pocket. Two models are used, the solvated protein and the protein in a solvated POPC lipid bilayer. We apply homology modeling, classical molecular dynamics simulations, density functional theory (DFT), and quantum mechanical/molecular mechanical (QM/MM) methods. Our results for the wild type bovine and human rhodopsins, used as a reference, are in good agreement with experiment. For the mutants, we find less twisted QM/MM ground-state chromophore geometries around the C(11)-C(12) double bond and substantial blue shifts in the lowest vertical DFT excitation energies. An analysis of the QM energies shows that the chromophore-counterion region is less stable in the mutants compared to the wild type, consistent with recent protein folding studies. The influence of the mutations near the chromophore is discussed in detail to gain more insight into the properties of these mutants. The spectral tuning is mainly associated with counterion effects and structural features of the retinal chain in the case of the hM207R mutant, and with the presence of a neutral chromophore with deprotonated Lys296 in the case of the hS186W mutant. 