

Cuban Research in Current International Journals

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

De Marco Formula effectiveness as an adjunctive therapy to prevent infected ischemic diabetic foot amputation and reduce plasma fibrinogen. Mesa MG, Duarte HA, Carretero JH, Fors López MM, Vilas MM. *J Tissue Viability*. 2011 Jan 4 [Epub ahead of print]

Background De Marco Formula (DMF) is a new procaine chemical combination of Procaine HCl and polyvinylpyrrolidone. A prospective randomized controlled clinical trial demonstrated that infected ischemic diabetic foot treatment with DMF for 52 days as an adjuvant with conventional therapy reduced major amputations. **Objective** To evaluate the possible association of clinical effectiveness and plasma fibrinogen reduction with DMF therapy. **Methods** Adult patients, 24 male/23 female, suffering from infected ischemic diabetic foot ulcers were randomly assigned to receive conventional therapy alone (group A, N = 24) or combined with DMF (receiving 0.15 ml/kg day i.m.) for ten days and then twice a week until lesion healing or completion of 52 days (group B, N = 23). Fibrinogen concentrations were determined before and after a ten-day treatment period. Treatment clinical responses were considered favorable if major amputations were not needed. Pre and post-treatment fibrinogen values were compared within each group and between groups. Differences were considered statistically significant for $p < 0.05$. **Results** Fifty percent of group A patients (12/24) and 21.7% of the Group B (5/23) showed unfavorable responses (a 56.6% reduction for group B). There were not statistical differences between pre and post-treatment fibrinogen within Group A (406.7 ± 49.08 vs. 354.6 ± 62.5 , $p = 0.11$). However, post-treatment values were significantly lower within Group B (298.9 ± 15.24 vs. 487.1 ± 49.08 , $p = 0.0016$). Patients who showed favorable responses had statistically lower fibrinogen concentrations than those with unfavorable responses (280 ± 5.1 vs. 310 ± 7.1 , $p = 0.002$) within group B. **Conclusions** DMF combined with conventional therapy for infected ischemic diabetic foot was associated with plasma fibrinogen decrease.

Dengue: a continuing global threat. Guzmán MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. *Nat Rev Microbiol*. 2010 Dec;8(12):S7–S16.

Dengue fever and dengue haemorrhagic fever are important arthropod-borne viral diseases. Each year, there are ~50 million dengue infections and ~500,000 individuals are hospital-

ized with dengue haemorrhagic fever, mainly in Southeast Asia, the Pacific and the Americas. Illness is produced by any of the four dengue virus serotypes. A global strategy aimed at increasing the capacity for surveillance and outbreak response, changing behaviours and reducing the disease burden using integrated vector management in conjunction with early and accurate diagnosis has been advocated. Antiviral drugs and vaccines that are currently under development could also make an important contribution to dengue control in the future.

Depressive symptoms evaluated by the Calgary Depression Scale for Schizophrenia (CDSS): Genetic vulnerability and sex effects. Martín-Reyes M, Mendoza R, Domínguez M, Caballero A, Bravo TM, Díaz T, et al. *Psychiatry Res*. 2010 Dec 31.

The present study compares the occurrence of depressive symptoms evaluated by the Calgary Depression Scale for Schizophrenia (CDSS) in patients of Multiplex (MS) and Simplex Schizophrenia families (SS). The Positive and Negative Syndrome Scale (PANSS) was used to evaluate psychopathology. A total of 206 paranoid schizophrenia patients were studied according DSM-IV criteria. The Family Interview for Genetic Studies (FIGS) was used to study the families. A result in the FIGS for a positive family history of schizophrenia was referred as MS (patients); its lack as SS (patients). CDSS scores were compared among MS and SS patients and possible sex differences intra- and inter-groups were explored. In the analysis of our sample (30) 19% of the total persons with schizophrenia group was depressed. The depressive symptoms measured by the CDSS were higher in females and the MS males group. Males from MS group showed more depressive symptoms than males from SS group. No differences with females from both groups were found. Findings in this study underscore the importance of gender and family history in understanding the heterogeneity of schizophrenia. This study suggests that sex and familiar history is an important point for studying depressive symptoms.

Diagnosis and Screening for Cytomegalovirus Infection in Pregnant Women in Cuba as Prognostic Markers of Congenital Infection in Newborns: 2007–2008. Kourí V, Correa CB, Verdasquera D, Martínez PA, Alvarez A, Alemán Y, et al. *Pediatr Infect Dis J*. 2010 Dec;29(12):1105–10.

Background Human cytomegalovirus (HCMV) has established itself as the most

significant cause of congenital infection in the developed world. The objective of this research was prenatal identification of pregnant women at risk for developing active infection due to HCMV as well as to diagnose congenitally infected newborns. **Methods** A diagnostic algorithm based on specific immunoglobulin G (IgG), IgM, and, IgG avidity was used to screen serum from 1131 pregnant women enrolled prospectively from three municipalities from Havana City, Cuba during 2007–2008. Qualitative multiplex nested PCR and quantitative real time-based PCR testing for HCMV DNA were performed on urine and saliva specimens from women detected with active infection and from their newborns. **Results** Most women were seropositive to HCMV (92.7%), with 2.38% (27 women) having active infection. Primary infection was detected in 20 pregnant women (1.77%) while 7 patients (0.62%) had active nonprimary infection. HCMV DNA was detected in specimens from 9 of the 27 pregnant women by both PCR methods. HCMV congenital infection was diagnosed in 12 (1.06%) of the 26 live children born from 25 mothers with active infection, for a vertical transmission rate of 46.2%. Two fetal deaths were reported from 2 women with active infection; furthermore 2 newborns were symptomatic at birth and 2 showed sequelae during the follow-up done until 6 months of age. **Conclusions** Mothers with active infection during the pregnancy and with HCMV excretion had significant risks, RR = 1.16 and RR = 1.35, respectively, to have congenitally infected children.

Ethical Dilemmas in Genetic Testing: Examples from the Cuban Program for Predictive Diagnosis of Hereditary Ataxias. Mariño TC, Armiñán RR, Cedeno HJ, Mesa JM, Zaldivar YG, Rodríguez RA, et al. *J Genet Couns*. 2011 Jan 25. [Epub ahead of print]

Predictive testing protocols are intended to help patients affected with hereditary conditions understand their condition and make informed reproductive choices. However, predictive protocols may expose clinicians and patients to ethical dilemmas that interfere with genetic counseling and the decision making process. This paper describes ethical dilemmas in a series of five cases involving predictive testing for hereditary ataxias in Cuba. The examples herein present evidence of the deeply controversial situations faced by both individuals at risk and professionals in charge of these predictive studies, suggesting a need for expanded guidelines to address such complexities.

Hospitalization of Cuban children for giardiasis: a retrospective study in a paediatric hospital in Havana. Escobedo AA, Almirall P, Alfonso M, Salazar Y, Avila I, Cimerman S. *Ann Trop Med Parasitol.* 2011 Jan;105(1):47–56.

The medical records of the 185 children who, in 2007, were admitted to the Academic Paediatric Hospital 'Centro Habana', in the Cuban capital of Havana, because of giardiasis were analysed retrospectively. A standardized form was used to collect data on the socio-demographic characteristics, clinical features, laboratory diagnosis, treatment and length of stay of each child. Information on the 15 children who had incomplete medical records was excluded from the data analysis. Of the remaining 170 children, 85 (50.0%) were aged 1–4 years, 97 (57.1%) were male, and 106 (62.4%), 92 (54.1%) and 69 (40.6%) had presented with diarrhoea, vomiting, and/or abdominal pain, respectively. Most (91.2%) of the cases had been diagnosed by the microscopical examination of a duodenal aspirate, and the drugs that had been most used frequently were quinacrine and tinidazole, which had been given to 72 (42.4%) and 62 (36.5%) of the cases, respectively. The mean length of hospital stay was 4.9 days. Such information on the clinical characteristics of giardiasis among children living in an endemic area may be valuable to paediatricians and public-health officials who wish to screen for the disease.

Increased serum S-100B and neuron specific enolase—Potential markers of early nervous system involvement in essential hypertension. González-Quevedo A, García SG, Concepción OF, Freixas RS, Sotolongo LQ, Menéndez MC, et al. *Clin Biochem.* 2010 Dec 1.

Objectives To investigate the occurrence of sub-clinical neurologic involvement in patients with essential hypertension employing serum biochemical markers. **Design and methods** Fifty patients with essential hypertension and 42 controls with no clinical evidence of neurological disease were recruited. Serum S-100B protein and neuron specific enolase (NSE) were determined by employing immunoassay kits from CanAg Diagnostics AB (Sweden). Brain MRI and fundoscopic exploration were conducted. **Results** S-100B and NSE levels were significantly higher in hypertensive patients than in controls. In hypertensive patients, multivariate analysis revealed that NSE was independently associated with two variables expressing severity of hypertension: diastolic blood pressure and grade of retinopathy. Brain MRI studies demonstrated higher NSE levels in patients with more severe white matter lesions. **Conclusions** Raised NSE levels are associated with a higher severity of hypertension and of white matter lesions, providing preliminary evidence that suggests the presence of silent brain damage in a subset of hypertensive patients.

Long-term persistence of clinical symptoms in dengue-infected persons and its association with immunological disorders. García G, González N, Pérez AB, Sierra B, Aguirre E, Rizo

D, et al. *Int J Infect Dis.* 2011 Jan;15(1):e38–43. **Objectives** The acute manifestations of dengue are well known. The clinical symptoms that present during the convalescent phase of infection are less well characterized, but may be autoimmune-based. This study was undertaken to determine the prevalence of persistent clinical symptoms among individuals infected during the 2006 Cuban epidemic and to evaluate the immunological and genetic factors associated with their occurrence. **Methods** In 2008, clinical data and blood samples were collected from a random sample of adult individuals diagnosed during the 2006 epidemic with dengue fever (DF, n = 68), dengue hemorrhagic fever (DHF, n = 29), or an asymptomatic infection (AI, n = 42). The presence of persistent symptoms was evaluated in all individuals and a psychological assessment was performed. IgG titers and the Fc receptor (FcR) were also evaluated. The following autoimmune markers were assessed in a subset (n = 26) of symptomatic individuals: complement factors C3/C4, rheumatoid factor (RF), C-reactive protein (CRP), antinuclear antibodies (ANA), and immune complex (IC). **Results** Over half (55/97) the individuals with a prior diagnosis of DF or DHF had persistent clinical symptoms in the 2 years following infection. The sequelae were unrelated to the initial diagnosis and were more common among women (44/55). No symptoms were reported in the AI group and all study participants had normal mental and cognitive function. Persistent clinical symptoms were associated with HH polymorphic variant (p = 0.027) and high IgG titer (p = 0.041). Autoimmune marker alterations were common (20/26) in the subset of symptomatic individuals evaluated. **Conclusions** Clinical sequelae after recovery from an acute dengue virus infection are common in the 2 years following infection. The results obtained in this study suggest that persistent symptoms are associated with alterations in some immunological parameters and FcγRIIIa gene polymorphism. This could suggest an autoimmune-based disturbance.

Newborn hearing screening test with multiple auditory steady-state responses. Mijares Nodarse E, Herrera Alonso D, Gaya Vázquez J, Santos Febles E, Cecilia Pérez Abalo M, Menéndez Alarcón L, et al. *Acta Otorrinolaringol Esp.* 2011 Jan 5. [Epub ahead of print]

Introduction The techniques most frequently used within a screening context (otoacoustic emissions and click auditory brainstem response) have well-known limitations in hearing loss detection. **Objective** This study examines the feasibility of a semi-automated multiple auditory steady-state responses (MSSR) system designed for neonatal hearing screening. **Methods** A sample of 50 newborns without risk factors (well-babies) was tested within two weeks of birth. All had detectable auditory brainstem responses to clicks down to 40 dB nHL in both ears. Two amplitude modulated carrier tones of 500 and 2,000 Hz were mixed together and presented simultaneously. Each infant (and ear) was screened with the MSSR system; to simu-

late a hearing loss, a recording without stimulation was also obtained. **Results** Mean auditory thresholds were 42.5±7 dB HL at 500 Hz and 35.5±6 dB HL at 2,000 Hz. The average duration of the MSSR recording was 2.6±1.6 minutes for each tested ear and the overall duration of the screening procedure (including electrode fitting and infant preparation) was 17.8±3.7 minutes. The diagnostic sensibility and the positive predictive values of the MSSR semi-automatic screening system was 100% and 96% respectively, with specificity of 96% and negative predictive values of 100%. **Conclusions** Although the diagnostic efficiency of the semi-automated MSSR system was found adequate, further technological improvements are still necessary to facilitate its use in the context of universal newborn hearing screening program.

Process-oriented fidelity research assists in evaluation, adjustment and scaling-up of community-based interventions. Pérez D, Lefèvre P, Castro M, Sánchez L, Toledo ME, Vanlerberghe V, et al. *Health Policy Plan.* 2010 Dec 12.

Fidelity research can help to answer essential questions about the diffusion process of innovative health interventions and provide insights for further scaling-up and institutionalization. This study assessed fidelity and reinvention in the implementation of a community-based control strategy for *Aedes aegypti* control. The intervention was implemented in 16 study areas in La Lisa, a municipality of Havana, Cuba. Its major components were: organization and management, capacity-building, community work and surveillance. A participatory assessment of process data was performed to determine whether the components and subcomponents were implemented, not-implemented or modified. Frequencies were tabulated over all circumscriptions (lowest level of local government) and the average was calculated for the four components. Spearman Rank correlation coefficients were calculated to explore the relationships between components. In addition, semi-structured interviews were conducted with coordinators of the strategy at different levels to identify difficulties encountered in the strategy's implementation. Surveillance was the most implemented component (72.9%) followed by capacity-building (54.7%). Community work and organization and management were less implemented or modified (50% and 45%, respectively). Apart from surveillance and capacity-building, all components are significantly and strongly correlated (Spearman Rank correlation coefficient was > 0.70, p < 0.01). If one component is implemented in a circumscription, the other components are also likely to be implemented. It is noticeable that areas which did not undergo organizational changes commonly did not implement community work activities. Within the whole strategy, few activities were added. Scarcely implemented sub-components were the most innovative. The difficulties encountered during implementation were related to appropriate training and skills, available time, lack of support and commitment

to the strategy, lack of motivation of local leadership, and integration of actors and resources. The study showed a wide variability of fidelity in the implementation of the intervention and highlighted challenges for scaling-up and institutionalization of the community-based intervention.

Quantitative ultramicrotest for newborn screening of galactosemia in Cuba. Frómata A, Reyes EC, Castells E, Tejeda Y, Almenares P, Pérez PL. *J Perinat Med.* 2010 Dec 21.

Background To describe a simple, rapid, quantitative ultramicrotest (UMTEST) based on the fluorometric method introduced by Fujimura et al. adapted to an Ultra Micro Analytic System (SUMA) for the detection of total galactose (GAL) in dried blood specimens. **Methods** The assay uses 3 mm discs of dried blood on Whatman 903 filter paper and small volumes of each reagent. A methanol/acetone/water solution is used for deproteination, and a specially designed 96-well polystyrene opaque ultramicroplates, with a maximum capacity of 30 µL per well, are used for the reading. **Results** The UMTEST GAL is completed in 2 h, with measuring range of 0.28–3.92 mmol/L. The intra- and inter-assay coefficients of variation were 2.3%–8.9% and 6.8%–11.1%, respectively, depending on the total GAL concentrations. Percentage recovery ranged from 97.7% to 103%. Limit of detection and limit of quantitation were 0.06 and 0.16 mmol/L, respectively. The mean GAL concentration, in 2510 dried blood samples from the National Neonatal Screening Program was 0.23 mmol/L. Our assay showed high concordance correlations with the commercially available ICN Immuno-Chem™ GAL-MW EA kit. **Conclusions** The analytical performance characteristics of this assay is suitable for mass newborn screening of galactosemia in Cuba.

Risk factors for *Giardia* infection among hospitalized children in Cuba. Bello J, Núñez FA, González OM, Fernández R, Almirall P, Escobedo AA. *Ann Trop Med Parasitol.* 2011 Jan;105(1):57–64.

The risk factors associated with *Giardia* infection, in children hospitalized in Havana, Cuba, were recently explored. Children aged ≥5 years were more likely to be positive for *Giardia* infection than the younger children, with an odds ratio (OR) of 3.41 [95% confidence interval (CI) = 1.36–9.69]. The risk factors found to be associated with *Giardia* infection in univariate analyses were rural residence (OR = 3.01; CI = 1.23–7.35), belonging to a household that did not receive water from an aqueduct (OR = 3.27; CI = 1.21–8.91), drinking unboiled water (OR = 3.64; CI = 2.14–6.26), nail biting (OR = 3.47; CI = 1.97–6.08), eating unwashed vegetables raw (OR = 4.84; CI = 2.33–10.14), and a personal (OR = 3.23; CI = 1.58–6.59) or family history (OR = 3.96; CI = 1.53–10.47) of previous parasitic infection. In multivariate analyses, however, only two (modifiable) risk factors were found to

be independently and significantly associated with *Giardia* infection: nail biting and eating unwashed vegetables raw. It therefore seems that, at least at the individual level, giardiasis-prevention activities in Havana should be focussed on health education to improve personal hygiene and food-related practices. If appropriately managed, the surveillance of drinking water and foodstuffs, for *Giardia* and other parasites, might also help to reduce the hospitalization of Cuban children.

Seroprevalence of antibodies to hepatitis E virus in two large communities in Havana, Cuba. Villalba MC, Guan M, Pérez A, Corredor MB, Frometa SS, Moreno AG, et al. *Trans R Soc Trop Med Hyg.* 2010 Dec;104(12):772–6.

Hepatitis E virus (HEV) infection is an important cause of acute viral hepatitis in tropical and subtropical regions that occurs both as epidemic episodes and sporadic cases. The aim of this investigation was to estimate the prevalence of total immunoglobulin (Ig) anti-hepatitis E virus (anti-HEV) and the risk factors associated to two communities in Havana City. Serum samples (n = 469) obtained from healthy individuals with no history of viral hepatitis were screened for total anti-HEV by enzyme immunoassay (EIA). An overall prevalence of 10% (47/469) (95% CI; 7.52–13.19%) was obtained. Higher anti-HEV prevalence was found in the municipality of Marianao (12.1%) compared to Playa (7.1%). Analysis of risk factors revealed a significant association of total anti-HEV prevalence in age group 41–60 years old, with a risk ratio of 3.21 (95% CI: 1.09–9.7) (p = 0.01). No relation was found between anti-HEV and other variables such as gender, risk occupations, surgery and transfusions. The detection of total anti-HEV in individuals with no overt acute hepatitis suggests the existence of sub-clinical or anicteric HEV infection in these municipalities.

Study of Various Presentation Forms for a Peptide Mimetic of *Neisseria meningitidis* Serogroup B Capsular Polysaccharide. Garay H, Menéndez T, Cruz-Leal Y, Coizeau E, Noda J, Morera V, et al. *Bioconjug Chem.* 2011 Jan 19;22(1):33–41.

The formulation of a broadly protective vaccine to prevent the serogroup B *Neisseria meningitidis* (MenB) disease is still an unmet medical need. We have previously reported the induction of bactericidal and protective antibodies against MenB after immunization of mice with a phage-displayed peptide named 4 L-5. This peptide mimics a capsular polysaccharide (CPS) epitope in MenB. With the aim of developing vaccine formulations that could be used in humans, we evaluate in this study various forms of presentation to the immune system of the 4 L-5 sequence, based on synthetic peptides. We synthesized the following: (i) a linear 4 L-5 peptide, (ii) a multiple antigen peptide containing four copies of the 4 L-5 sequence (named MAP), which was then dimerized, and the product named dimeric MAP, and (iii) a second multi-

ple antigen peptide, in this case with two copies of the 4 L-5 sequence and a copy of a T-helper cell epitope of tetanus toxoid, which was then dimerized and the product named MAP-TT. The linear peptide, the MAP, and the dimeric MAP were conjugated to the carrier protein P64K by different conjugation methods. Plain antigens and antigens coupled to P64K were used to immunize BALB/c mice. Of those variants that gave immunogenic results, MAP-TT rendered the highest levels of specific anti-peptide IgG antibodies and serum bactericidal activity. These results can find application in the development of meningococcal vaccine candidates and in peptide-based vaccines strategies.

Virus Role During Intraepidemic Increase in Dengue Disease Severity. Rodríguez-Roche R, Sanchez L, Burgher Y, Rosario D, Alvarez M, Kouri G, et al. *Vector Borne Zoonotic Dis.* 2011 Jan 22. [Epub ahead of print]

Dengue epidemics in Cuba have repeatedly demonstrated a month-to-month increase in clinical severity during secondary infections. The dengue 2 outbreak that occurred in Santiago de Cuba in 1997 was accompanied by the most severe intraepidemic increase in disease severity reported to date. It was initially proposed that the appearance of neutralization escape mutants during the course of the epidemic might explain this phenomenon. Recent studies have revealed that during the course of this epidemic, nucleotide substitutions appeared only in nonstructural (NS) genes, most of which were silent, except for one change in the NS1 gene. To study whether or not variation in the NS1 gene might be associated with increased disease severity during the epidemic, this gene was partially sequenced from 15 isolates obtained at different times during the 1997 epidemic. Early epidemic isolates differed from those obtained later by replacement only of threonine with serine at position 164 in the NS1 protein, an amino acid rarely found in any genotype of dengue 2 virus. All viruses isolated from patients located in Health Districts, where dengue 2 transmissions occurred late in the epidemic, contained serine at position 164, indicating that this change was fixed within a few months. Here we argue that this single mutation contributes to viral survival or replication efficiency, resulting in enhanced infection in the presence of enhancing antibodies, a phenomenon that we term increased virus “fitness” in contrast to “virulence,” an intrinsic property of the virus. 