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SUMMARY

AREA: Biological Sciences
SUBAREA: Biotechnology

DO DIFFERENT FOOD BIOACTIVE COMPOUNDS PROMOTE THE *IN VITRO* EFFECTS OF CURRENT IMMUNO/CHEMOTHERAPY FOR COLORECTAL CARCINOMA?

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Colorectal carcinoma (CRC) has a poor prognosis, and current treatment strategies, including 5-fluorouracil (5-FU) and irinotecan (CPT-11) chemotherapy or 5-FU and bevacizumab (BVZ) immunotherapy *doublets*, do not yield desired outcomes. Therefore, exploring alternative drug combinations is encouraged, including the use of food-derived bioactive compounds (BC), which antitumor potential remains unexplored. Hence, we evaluated whether BC enhance the antitumor effects of current chemo/immunotherapy regimens for CRC *in vitro*. Spheroids (3D) of HCT-116 CRC cells and CCD-18Co colonic fibroblasts (ultra-low adherence plates, in 2:1 ratio), or HCT-116 monolayers (2D) were individually treated with allyl (AITC) or phenethyl (PEITC) isothiocyanates, capsaicin (CPS), chlorogenic acid (CGA), BVZ, CPT-11, or 5-FU to determine the maximal effective concentration (EC_{50}) by MTT assay (24, 48 h). In spheroids, 1/5 or 1/10 EC_{50} of PEITC (8.8 and 4.4μM), AITC (1513 and 757μM), CPS (79 and 39μM) or CGA (560 and 280μM) were combined with 5-FU+CPT-11 or 5-FU+BVZ *doublets* at 1/5 (1+30μM or 1+15μM) and 1/10 (0.5+15μM or 0.5+7.5μM) of the EC_{50} - similarly to plausible serum/tissue levels - and cell viability (MTT) was evaluated, and the Combinatory Index (CI, 1= additive, <1 synergism, >1 antagonism) was calculated. Only AITC promoted the BVZ+5-FU-induced reduction in spheroid viability and had synergistic effects in CI calculation (0.97), whilst it did not alter CPT-11+5-FU effects. Using an HCT-116:CCD-18Co transwell model (2D), 1/5 EC_{50} AITC (188μM) was further combined with 1/5 EC_{50} 5-FU+BVZ (11+7μM) to assess motility and migration after 36 hours. Only AITC+5-FU+BVZ reduced CCR cell motility, while it did not modify invasion. Data suggest that AITC promotes the antitumor effects of current immuno plus chemotherapy regimen for CCR, warranting further *in vitro* (RNA-Seq) and *in vivo* (cell-derived xenograft) investigation.

KEYWORDS: Chemotherapy; colorectal carcinoma; immunotherapy; isothiocyanate.
FUNDING: São Paulo Research Foundation (#22/06082-8; #24/06431-8; #24/09284-6).

AREA: Health Sciences
SUBAREA: Medicine

FULL-SPECTRUM *CANNABIS* EXTRACTS PROMOTES ACTIVE COPING IN A NOVEL, AI-DRIVEN ZEBRAFISH MODEL OF POST-TRAUMATIC STRESS DISORDER

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Post-Traumatic Stress Disorder (PTSD) is a major clinical challenge requiring robust translational models to develop new pharmacotherapies. Zebrafish (*Danio rerio*) are a valuable neurobehavioral model, but there is a gap in models that capture the complexity of PTSD. To explore how acute trauma is triggered by specific visual cues, our objective was to develop and validate an AI-powered zebrafish PTSD model and to assess the therapeutic potential of chronic treatment with a full-spectrum Cannabis oil, investigating the "entourage effect."

The study involved two experiments. First, for validation, zebrafish were divided into control and stressed groups, with the latter subjected to an acute, multimodal stress protocol paired with a red visual cue. Second, for pharmacological testing, a new cohort underwent the same stress protocol while receiving a chronic diet with full-spectrum Cannabis oil (equivalent to 10 mg/kg/day of CBD). Behavior was monitored for seven days using a custom AI tracking system (YOLO).

All procedures were approved by the UNESP Ethics Committee (protocol no. 4806060624). The stress protocol successfully induced a persistent PTSD-like phenotype, including locomotor retardation, increased freezing, and a strong aversive memory—shifting the innate preference for the red cue to active avoidance. Chronic treatment with the full-spectrum oil reversed the trauma-induced lethargy but did not eliminate the fear memory; instead, it modulated behavior, shifting the coping strategy from passive freezing to active avoidance.

The AI-based paradigm proved to be a high-precision platform for assessing core PTSD symptoms. The results suggest that the therapeutic effect of the Cannabis extract lies in modulating behavioral expression rather than suppressing fear memory. By reversing anhedonia-like states and promoting active coping, full-spectrum Cannabis oil fosters more adaptive responses to trauma triggers—an important translational insight.

In conclusion, this study establishes a novel translational PTSD model and provides evidence that full-spectrum Cannabis oil, through the "entourage effect," modulates the expression of traumatic memory, highlighting its potential as a promising pharmacotherapy for stress-related disorders.

KEYWORDS: PTSD; Zebrafish; Cannabidiol; Animal Model; Artificial Intelligence.

FUNDING: This research was supported by the São Paulo Research Foundation (FAPESP), grant number 2023/14200-3.

ASSESSMENT OF THE CYTOTOXIC POTENTIAL OF HIGH- AND LOW-MOLECULAR-WEIGHT COMPONENTS FROM *CROTALUS DURISSUS TERRIFICUS* VENOM AGAINST HEPG2 TUMOR CELLS

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Introduction: Hepatocellular carcinoma (HCC) is a highly lethal malignancy with rising global incidence and limited therapeutic options. Given the urgent demand for novel anticancer agents, bioactive compounds from animal venoms have gained attention. *Crotalus durissus terrificus* (Cdt) venom comprises a diverse set of proteins and peptides with recognized cytotoxic potential. While high-molecular-weight toxins like crotoxin and PLA_2 are well studied, low-molecular-weight peptides remain largely unexplored. This study investigates the cytotoxic effects of both fractions on HepG2 cells to identify novel venom-derived antitumor candidates.Objective: To characterize, through chromatographic profiling, and evaluate in vitro the cytotoxic effects of high- and low-molecular-weight fractions from Cdt venom on HepG2 hepatocellular carcinoma cells.Methodology: Crude Cdt venom from CEVAP-maintained specimens was fractionated by ultrafiltration (3 kDa cut-off). Fractions were analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC). HepG2 cells were cultured in DMEM with fetal bovine serum and exposed to increasing concentrations of each fraction. Cell viability was measured using the MTT assay after 24 and 48 hours. CCCP was used as a positive control. Data were analyzed statistically.Results: RP-HPLC confirmed effective molecular separation, with distinct chromatographic profiles for high (>3 kDa) and low (≤3 kDa) mass fractions. Both exhibited significant cytotoxicity toward HepG2 cells. Low-mass peptide fraction (2,000–3,000 μg/mL) and high-mass compounds (500–1,000 μg/mL) reduced viability after 48 h ($p<0.05$), with reproducible results across replicates.Discussion: Findings demonstrate that both venom fractions possess potent cytotoxic effects on HepG2 cells. The magnitude of cell viability reduction suggests interference with key metabolic pathways, likely involving mitochondrial and membrane disruption. Although activity was slightly lower than in previous reports—possibly due to solvent absence and sample handling—cytotoxicity remained robust, underscoring the therapeutic potential of these fractions.Conclusion: High- and low-molecular-weight fractions from *Crotalus durissus terrificus* venom exhibit notable cytotoxic activity against HepG2 cells. These results support further investigation into their isolation, structural characterization, mechanisms of action, and preclinical validation as potential anticancer agents for HCC.

KEYWORDS: Snake venom; peptides; anticancer agent; hepatocellular carcinoma; HEPG2 cells.
FUNDING: Fapesp 2021/11936-3

IMMUNOMODULATION WITH B-GLUCANS INFLUENCES THE EXPRESSION OF PLASMA CHEMOKINES AFTER PRIMARY VACCINATION FOR COVID-19

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Introduction: Various strategies are employed to enhance vaccine efficacy including the use of immunomodulators such as β-glucans. These natural biomolecules are polysaccharides derived from the cell walls of yeast or bacteria. They are capable of promoting trained immunity (TRIM), a mechanism that activates innate immune cells and induces a more vigorous and effective secondary immune response. Objective: To evaluate the immunomodulatory capacity of orally administered β-glucans on chemokine production following primary vaccination against SARS-CoV-2 with the ChAdOx1 nCov-19 vaccine. Materials and methods: 28 unvaccinated men naive to SARS-CoV-2 were evaluated and divided into two groups according to the supplement administered for 14 consecutive days (starting 7 days before the first vaccine dose): Glucan (n=16) and control (n=12). Peripheral blood samples were collected at three time-points: pre-vaccination (PV), 30 days after each vaccine dose, 1st dose (1D), and 2nd dose (2D). Plasma levels of the chemokines IL-8, RANTES, MIG, MCP-1, and IP-10 were quantified using the CBA Human Chemokine Kit® (BD) by flow cytometry. Research Ethics Committee of UNESP-opinion nº.6.589.361. Results: No significant differences were observed in the comparative analyses between the groups. However, a significant increase in IP-10 (p=0,0216) and RANTES (p=0,0202) was observed in the glucan group after 1D in the paired evaluation of timepoints from the same individual, as well as an increase in MIG after 2D (p=0,386). IL-8 decreased after 2D (p=0,0413). In the control group a reduction in IL-8 was evident at 1D (p=0,004883) compared to PV. The other variables did not differ significantly. Discussion: Chemokines act by modulating innate/adaptive immune responses, and their balance is essential for establishing protective immunity. IP-10, elevated only in the Glucan group and synthesized by monocytes, is directly linked to CD4+T lymphocytes, and is related to how innate immune response cells activate adaptive immune response cells, as well as themselves (feedback), leading to the production of other inflammatory cytokines and long-lasting memory T cells, which are desirable in vaccination. Conclusion: In this context, it is believed that β-glucan supplementation prepares the immune system to receive the vaccine, promoting cellular immunomodulation, such as monocytes and T cells, inducing greater production of chemokines, and possibly targeted and long-lasting cellular memory.

KEYWORDS: chemokines; β-glucans; SARS-CoV-2; trained immunity; vaccine.
FUNDING: PROPG-UNESP.

AREA: Health Sciences
SUBAREA: Medicine

CHARACTERIZATION OF THE IMMUNE-INFLAMMATORY RESPONSE IN EXPERIMENTAL EXTRAPARENCHYMAL NEUROCYSTICERCOSIS AT DIFFERENT TIMES OF INFECTION

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Abstract: Neurocysticercosis, caused by ingestion of *Taenia solium* eggs, is the most prevalent zoonotic infection of the central nervous system. The parasite evades host immunity by inducing a Th2-skewed response, allowing persistence. Whether the inflammatory profile changes over time in an experimental *Taenia crassiceps* model remains unclear. Objective: To characterize the temporal profile of the inflammatory response in a rat model of extraparenchymal neurocysticercosis induced by *T. crassiceps*. Methods: Thirty-three *Wistar* rats were inoculated intracranially with *T. crassiceps* cysts and euthanized at 1, 3, or 6 months post-infection; five non-infected rats served as controls. Peripheral blood was tested for anti-*T. crassiceps* IgG. Brains underwent morphometry, immunohistochemistry for GFAP and Iba-1, and ELISA for IL-2, -4, -5, -6, -8, -10, -17, TNF-α, TGF-β, and IFN-γ. Results: Morphometry revealed progressive ventricular dilation, most pronounced at 6 months. GFAP and Iba-1 expression increased over time. At 3 months, IL-5 ($p=0.0008$) and IL-17 ($p=0.0006$) were elevated, while IL-8 decreased ($p=0.0335$) versus controls. IL-6 declined at 3 ($p=0.0323$) and 6 months ($p=0.0258$) compared to 1 month, which was elevated relative to controls ($p=0.0013$). IL-8 decreased at 3 months versus 1 month ($p=0.0035$). IL-17 rose at 1 month ($p=0.0015$) versus controls. TGF-β increased at 3 months versus 1 month ($p=0.0052$). Other cytokines remained stable. All infected groups showed IgG seropositivity, with higher levels and avidity from 3 months onward. Conclusion: The infection triggers a sustained, chronic inflammatory process that is initially robust but progressively shifts toward a more permissive profile, accompanied by glial activation and morphological damage.
KEYWORDS: neurocysticercosis; inflammation; immune response; animal model.
FUNDING: FAPESP - 2023/12860-6.
Ethic statement: CEUA 1426/2023

SMALL-VOLUME DOSE INACCURACY IN LIQUID VALPROATE: BRIDGING THE GAP BETWEEN DOSE DELIVERY AND CLINICAL PRACTICE

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Introduction: Sodium valproate is an anticonvulsant commonly prescribed in liquid form to pediatric patients or those with neurological impairments, owing to the ease of administration of this dosage form. However, the accuracy of small-volume liquid dose measurements remains uncertain and clinically relevant, particularly for drugs acting on the central nervous system, such as valproate. Objective: To investigate the variation in valproic acid content in small, medium, and large doses of valproate syrup when measured using the dosing cup, and to discuss its implications. Methodology: Doses of 2.5 mL / 125 mg, 5 mL / 250 mg, and 7.5 mL / 375 mg of valproate syrup were measured using the marked dosing cup provided with the product (n = 10). Valproic acid content was quantified by high-performance liquid chromatography with UV detection, in accordance with the United States Pharmacopeia (specification: 90–110% of the labeled content of valproic acid). Results: The mean valproic acid content was 81.8% for 2.5 mL (RSD = 17.2%), 88.3% for 5 mL (RSD = 9.6%), and 93.1% for 7.5 mL (RSD = 9.0%). A significant difference was observed between the 2.5 mL and 7.5 mL doses ($p = 0.026$). Notably, the valproic acid content in the syrup itself was 97%, within acceptable pharmacopeial limits. Almost all 2.5 mL and 5 mL doses were underdosed (< 90% of valproic acid), whereas 7.5 mL doses were generally within acceptable limits and showed less variability. Discussion: These findings reveal a potential source of therapeutic failure not related to drug potency, but to formulation design and administration practices. Small-volume measurements with dosing cups may result in clinically significant underdosing. This problem is magnified in vulnerable populations, such as pediatric patients, and remains insufficiently addressed in both pharmaceutical development and regulatory oversight. Conclusion: This study underscores a translational gap between dose delivery and clinical practice. Improving dose accuracy for liquid medications, particularly those with psychoactive properties, is essential to ensure therapeutic efficacy and patient safety. Innovative approaches for designing user-friendly, accurate, and standardized dosing devices—especially for low-volume administration—are urgently needed to support safe pharmacotherapy.

KEYWORDS: Dose accuracy; clinical applicability; dosing device; liquid medication; therapeutic failure.

CONSTRUCTION AND VALIDATION OF A NAÏVE VHH PHAGE DISPLAY LIBRARY

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Antibodies are essential proteins involved in the detection of foreign agents, characterized by their high specificity and broad applicability in diagnostics and therapies. Among their variants, heavy-chain antibodies (HCAbs), found in camelids such as llamas and alpacas, possess a variable domain (VHH) of approximately 15 kDa as their antigen-binding region. These VHH domains, also known as nanobodies, offer several advantages over conventional antibodies, including reduced size, high solubility, stability under extreme conditions, and the potential for bacterial expression. Since the approval of the first nanobody-based drug, Caplacizumab, in 2019, interest in the development of these molecules has been increasing. Among the available strategies, the construction and selection of naïve *phage display* libraries has stood out for enabling the rapid isolation of target-specific antibodies while mimicking the natural immune repertoire of these animals. The *phage display* technique is robust, accessible, and efficient for the identification of novel therapeutic targets. In this context, the present study aims to implement the construction of a naïve VHH library displayed on phages, with the future goal of generating therapeutic candidates. For this purpose, blood samples were collected from four non-immunized llamas (CEUA 000.373/2025), followed by the isolation of lymphocytes via density gradient centrifugation. Total RNA was extracted and converted into cDNA, and VHH genes were amplified by nested PCR. The resulting fragments were cloned into the pCOMB3XSS vector through SfiI digestion and ligation using T4 DNA ligase. Aliquots of electrocompetent *Escherichia coli* were transformed by electroporation, yielding 6.39×10^7 transformants. Sanger sequencing analysis revealed 100% clonal diversity, resulting in an actual library size of 1.66×10^7 functional clones. Selection assays are still required to validate the library's capacity to identify specific binders. Despite promising results, recombination events were detected in the phagemid vector, leading to reduced cloning efficiency. Therefore, optimization of the cloning process will be recommended to improve efficiency. This project contributes to strengthening national capacity for the construction of antibody fragment libraries and paves the way for establishing a robust platform for developing libraries targeting pharmaceutical molecules with potential applications in biotechnology and healthcare.

KEYWORDS: Heavy Chain Antibodies; VHH; Nanobodies; Phage Display; Naïve Library.

FUNDING: The authors acknowledge financial support from the São Paulo Research Foundation (FAPESP, grant No. 2021/11936-3).

TECHNOLOGY, COMMUNICATION, AND PATIENT EXPERIENCE: ASSESSMENT AND REQUIREMENTS FOR INNOVATION FROM USERS' PERCEPTIONS IN URGENT AND EMERGENCY CARE SERVICES

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Introduction: Brazil's PNH and HumanizaSUS transform healthcare by centering Patient Experience (PX) and humanized management. Law No. 15,126 (Oct 29, 2025) legally integrates this into the SUS. Programs foster service integration, professional engagement, and quality care. Effective communication among patients, families, and professionals is crucial. Objectives: This project diagnoses communication in Botucatu Medical School's PSR HCFMB to enhance management and humanize PX. Based on user perceptions (patients, families, professionals), it identifies communication bottlenecks and improvement opportunities. It defines requirements and proposes a conceptual model for future real-time technology integrating traceability data (barcodes) for personalized notifications. This optimizes clinical information, reduces anxiety, and improves teamwork, fostering healthcare communication innovation. Methods: Design Thinking (exploration, creation, reflection, implementation) explores PSR patient experience challenges. Exploration includes value-stream analysis to identify communication bottlenecks, using VSM or existing flowcharts validated by participants. Data collection involves semi-structured interviews, questionnaires, and document analysis with patients, families, and professionals. This data informs developing and prototyping technological solutions for centralized, humanized communication. Expected Results: The study expects to identify main communication obstacles among patients, families, and healthcare teams via process analysis and stakeholder feedback. A Command Center prototype, integrated with an app, will centralize real-time clinical, operational, and communication information, fed by traceability data, triggering messages and alerts. It offers role-specific functionalities: informative messages for families/patients, clinical/operational guidance for nursing, and smart alerts for physicians (medical records/bedside documentation). The goal is transparent communication, reduced decision-action time, increased user satisfaction, and an integrated, patient-centered hospital environment. Discussion: Identified communication bottlenecks and proposed technological framework offer insights into humanizing urgent care. Integrating real-time traceability data with personalized notifications enhances patient experience, fostering transparency, anxiety reduction, and operational efficiency in high-complexity hospitals. This directly advances humanized healthcare and translational science. Conclusion: This project's key implication is a replicable conceptual model for a communication platform. It fosters an integrated, collaborative, patient-centered hospital ecosystem by optimizing information flow, shortening decision-action times, and increasing user satisfaction. This work contributes to technological innovation in healthcare, promoting effective communication as a cornerstone of quality patient care.

Keywords: Humanization of Healthcare; Health Communication; Patient Experience; Smart Hospital; Healthcare Technology.

Ethics: This study was approved by the Research Ethics Committee (CEP) of FMB-Unesp.

BROADLY NEUTRALIZING ANTIBODIES TARGETING THE SARS-COV-2 FUSION PEPTIDE VIA PHAGE DISPLAY

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Antibodies were key molecules for understanding and combating infectious agents such as SARS-CoV-2, with roles in viral neutralization, diagnostics, and immunotherapy, especially when vaccines were unavailable or ineffective. Most therapeutic antibodies had been developed against the Spike protein, but their efficacy declined due to antigenic variation, particularly mutations in the receptor-binding domain (RBD). This underscored the need for broadly neutralizing monoclonal antibodies (bnAbs) capable of targeting both current and future variants. The fusion peptide, a highly conserved element essential for viral entry, represented a promising target for therapeutic and preventive strategies. This project aimed to generate bnAbs from a hyperimmune phage-display library of scFv fragments constructed from infected and vaccinated individuals at Instituto Emílio Ribas and Instituto Adolfo Lutz, Brazil. Bioselection was directed toward the fusion peptide, and the most reactive antibodies were expressed in the scFv-Fc format, purified, and assessed for neutralization. Functional characterization highlighted activity against variants of concern (VOCs), particularly through microneutralization assays. The constructed scFv library comprised approximately 3×10^6 transformants, with a 95% success rate of insert incorporation among 64 randomly selected colonies. Restriction analysis with MvaI confirmed the high sequence diversity of the library. This work generated a diverse repertoire of neutralizing antibodies (nAbs) against SARS-CoV-2 variants, reinforcing their potential for the development of effective immunotherapies against COVID-19. Complementary activities were conducted at the Icahn School of Medicine at Mount Sinai (New York, USA).

KEYWORDS: broadly neutralizing monoclonal antibodies; SARS-CoV-2 fusion peptide; phage display technology; viral variants of concern; COVID-19 immunotherapy

FUNDING: This project is part of two ongoing initiatives: a research-abroad internship (BEPE) under a FAPESP-funded Ph.D. project (Grant 2022/05566-1) and the Center for Translational Science and Biopharmaceutical Development (FAPESP Grant 2021/11936-3).

CROSS-REACTIVITY POTENTIAL OF MENINGOCOCCAL OMVS FORMULATED WITH ALUM AND CHOLERA TOXIN B SUBUNIT IN NEONATAL, YOUNG AND ADULT MICE

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The development of safe and effective vaccines remains a challenge, particularly for children and young adults, who are the main risk groups for meningococcal disease. In Brazil, SUS coverage is limited for several serogroups, further constrained by strain variability and the high cost of recombinant vaccines, which hinders their incorporation into the national immunization schedule. *Neisseria meningitidis* outer membrane vesicles (OMVs), rich in antigens and highly immunogenic, emerge as a promising platform for vaccines adapted to the Brazilian epidemiological scenario. In this study, cross-reactivity among different *N. meningitidis* serogroups was evaluated using sera from BALB/c mice initially immunized in the neonatal phase with three subcutaneous doses of OMVs from the C:2a:P1.5 strain, formulated with aluminum hydroxide (Alum) and cholera toxin B subunit (CTB) (CTC/IAL 34-P/2023). IgG levels and avidity against the homologous C:2a:P1.5 strain were assessed by ELISA after three doses. Immunoblot was used to evaluate IgG specificity against antigens from the homologous strain, as well as cross-reactivity with B:4:P1.9 and B:4:nt strains, given that serogroup B is highly relevant in the Brazilian epidemiological context. Dot blot was employed to assess IgG reactivity against 67 heterologous strains from serogroups B, C, W, and Y circulating in 2023. High levels of IgG were detected ($p < 0.0001$), along with antibodies exhibiting strong affinity (76.35 ± 14.74). IgG antibodies recognized antigens of 25–58 kDa and 100–190 kDa in the homologous strain. Cross-reactivity was mediated by antigens of 17–80 kDa (B:4:P1.9) and 32–58 kDa (B:4:nt), indicating recognition of distinct antigens in heterologous strains, which do not share serogroup, subtype, or serotype with the vaccine strain. Immune sera reacted with different strains from serogroups B, C, and W. These results reinforce the potential of OMVs as a vaccine platform, whose antigenic diversity facilitates the recognition of strains with epidemiological relevance in Brazil, including the most prevalent serogroup B. The OMV–CTB–Alum formulation appears promising, as it induces an effective immune response, highlighting its potential to increase coverage given the diversity of circulating strains in the country. However, serum bactericidal assays would be required to confirm protection against *N. meningitidis* strains.

KEYWORDS: *Neisseria meningitidis*; OMVs; Cross-reactivity; Alum; Cholera Toxin subunit B

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HIGH AVIDITY IGE INDUCED BY SARS-COV-2 INFECTION AND VACCINATION

Oliveira, G.S.[1,2]; Portilho, A.I.[1,2]; Silva, V.O.[3,4]; Costa, H.H.M.[1,2]; Yamashiro, R.[1]; Oliveira, I.P.[5]; Campos, I.B.[6]; Prudencio, C.R.[1,2]; Matsuda, E.M.[5]; Brigido, L.F.M.[3,4]; Gaspari, E[1,2].

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IgE is traditionally associated with allergies and parasitic infections, but growing evidence suggests that viral antigens can also be IgE-binding ligands. The investigation of the IgE response to SARS-CoV-2 can offer new perspectives on viral immunity, contributing to translational science. The objective of this study was to determine whether COVID-19 infection and vaccination (CoronaVac, ChAdOx1, and BNT162b2) trigger a specific IgE response against the Receptor-Binding Domain (RBD). In a cohort of 59 volunteers, specific IgE to the RBD and its avidity were assessed by ELISA, with serum samples collected at three time points: pre-vaccination, after two doses (ChAdOx1 or CoronaVac), and after a booster dose (BNT162b2). IgG depletion experiments on 16 samples and pre-pandemic sera as controls were used to confirm the specificity of the IgE response (CAAE 31924420.8.0000.0059). COVID-19 infection caused a discrete increase in IgE, with intermediate avidity (Median; IQR25-IQR75) (51.19; 35.95-57.78). The initial two vaccine doses did not show a significant change in IgE levels. However, the booster dose resulted in a statistically significant increase in IgE levels ($p < 0.001$). The avidity of vaccine-induced IgE was high for both CoronaVac + BNT162b2 (76.64; 66.92-83.26) and ChAdOx1 + BNT162b2 (73.53; 59.61-81.64), with no statistical difference between them. IgG depletion confirmed that IgE detection was specific. Additionally, IgE levels and avidity correlated well with neutralization after the two doses of CoronaVac ($r = 0.7125$) or ChAdOx1 ($r = 0.7967$). This study establishes that SARS-CoV-2 infection and, notably, booster vaccination, are capable of inducing a specific IgE response. The high avidity of vaccine-induced IgE, along with its correlation with viral neutralization, suggests a potential functional role for IgE in the protective immune response. The absence of comorbidities related to the IgE response in the studied cohort, suggests this is part of the immune response to the virus, thus a point to be considered in future investigations, especially to elucidate its biological function.

KEYWORDS: IgE; COVID-19; Vaccines; Avidity

FUNDING: Fundação de Amparo à Pesquisa do Estado de São Paulo/FAPESP (grants numbers 2018/04202-0, 2018/14384-9, 2021/11936-3, 2022/05566-1); Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq (grants numbers 305301/2022-5, MS-DIAHV 24/2019 process 442776/2019-5); Financiadora de Estudos e Projetos (grant number 01160075); Fundo de Investimento para Educação Sanitária e Imunização em Massa contra Doenças Transmissíveis/FESIMA (grants numbers 011/2021, 59/2021) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/CAPES (finance code 001).

EVALUATION OF TWO INDEPENDENT VACCINE FORMULATIONS FOR *NEISSERIA MENINGITIDIS*: A TRANSLATIONAL STUDY

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Neisseria meningitidis, a gram-negative bacteria, is the etiologic agent of meningococcal disease (MD), a major concern worldwide. The bacteria is classified in serogroups according to its polysaccharide (PS) capsule, from which five (A, B, C, W, Y) are mainly associated with MD. Commercial vaccines, associating PS and recombinant proteins, are effective to prevent MD, but its cost restrain the accessibility of developing countries. Thus, the elderly are often misrepresented in vaccine trials, although MD incidence in this age group has increased. This work tests two new platforms, aiming to fulfill these challenges: affordable vaccine technologies and immunogenicity in the elderly. Outer membrane vesicles (OMVs) were extracted from the C:2a:P1.5 strain and used as antigen to immunize A/Sn (H2a) mice, and immunogenicity was evaluated through IgG, IgG1, IgG2a IgG2b, IgG3 levels (CTC/IAL 33P/2023). The first formulation used OMVs and Cholera toxin subunit B (CTB). Elderly mice were immunized with 0,8µg OMV+0,4µg CTB, via intranasal (IN) and intramuscular (IM) routes, with a booster 15 days after the prime. Control groups received OMV or CTB alone. The results show that the OMV+CTB (IN) and OMV+CTB (IM) were more immunogenic than controls,triggering an IgG response up to 5-8 times higher after the booster. Thus, the groups had similar outcomes for all isotypes analysed , exhibiting a mixed Th1/Th2 immune response, with stronger Th1 bias. The second formulation used OMV and PSC and PSA. Adult mice were immunized via IM route with three doses containing 5µg OMV+2.5µg PSA or +2.5µg PSC+0.1 mM/ml alum (AH), the control group received only AH. The results indicated that PSA was not immunogenic, even when used with OMVs, but PSC was immunogenic with OMVs.The IgG response were robust for OMV+PSC+AH, OMV+PSA+AH, OMV+PSA+PSC+AH and OMV+AH groups, triggering responses as high as 6-8-fold compared to controls, with a Th1 predominance. Both formulations seem promising, although OMV+CTB formulation seems more interesting, as the immune response from IN route was fairly similar to the IM one. IN immunizations have several advantages, such as simplified delivery, acceptance and local immunity. Nevertheless, it is necessary to expand these studies to ratify these findings with experiments such as ELISpot, to evaluate cellular response, and bactericidal activity, which is the gold standard for meningococcal vaccines.

KEYWORDS: OMV; vaccine; Neisseria meningitidis; polysaccharide; cholera toxin

FUNDING: Fundação de Amparo à Pesquisa do Estado de São Paulo/FAPESP (grants numbers 2018/04202-0, 2021/11936-3); Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq (grants number 305301/2022-5) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/CAPES (finance code 001).

EFFECTS OF FIBRIN BIOPOLYMER AND *CURCUMA LONGA* ON THE WOUND HEALING PROCESS IN THE SKIN OF RATS UNDERGOING OR NOT UNDERGOING PHOTOBIOMODULATION THERAPY

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Skin wound healing is a dynamic and complex process influenced by factors such as lesion size, local conditions, and systemic health. Impaired healing may lead to prolonged inflammation, increased risk of infection, and functional loss. The use of adjuvant therapies, such as biomaterials and photobiomodulation, has been studied as a strategy to enhance repair. This study aimed to evaluate the effects of local laser photobiomodulation (PBM), fibrin biopolymer (FB), and topical curcumin (CU) on the healing of full-thickness skin wounds in rats. Forty *Wistar* rats were divided into two groups: G1 (without PBM) and G2 (with PBM). Each animal received four standardized 8-mm circular wounds on the dorsum, treated respectively with: clot only (control), FB, CU, and FB combined with CU. Group G2 received PBM with red laser (660 nm, 1 J per point, three times per week). Animals were euthanized at 3, 7, 11, and 15 days post-injury for morphometric and histological analysis. FB-treated wounds showed smaller areas throughout the experimental periods and more advanced repair, with better epithelialization and collagen fiber organization compared to control. These effects were enhanced in G2 with PBM. CU-treated wounds presented delayed healing and less mature tissue organization, particularly when combined with FB. The data suggest that FB provided a favorable scaffold for cell migration and matrix deposition, contributing to faster tissue regeneration. PBM optimized these effects, likely by stimulating mitochondrial activity and enhancing cellular functions. Conversely, CU in the tested formulation showed no beneficial effect and, when associated with FB, appeared to interfere with healing progression. In conclusion, the combination of fibrin biopolymer and photobiomodulation promoted more effective skin wound healing in rats, whereas topical curcumin, as used in this study, was not advantageous and may have hindered repair. These findings highlight the potential of combining PBM and biomaterials such as FB for improving tissue regeneration.

KEYWORDS: wound healing; curcumin; photobiomodulation therapy; fibrin tissue adhesive

FUNDING: This study was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES – Brazil).

ETHICS: All procedures were approved by the Ethics Committee on the Use of Animals of the Bauru School of Dentistry, University of São Paulo (CEUA-FOB/USP), under protocol number 009/2016.

MOTOR NEURON PRESERVATION AND RECOVERY ARE IMPROVED AFTER EXPERIMENTAL NERVE REPAIR WITH HETEROLOGOUS FIBRIN BIOPOLYMER

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Introduction: Peripheral nerve injuries often result in incomplete recovery despite standard neurorrhaphy. This is due to spinal cord degeneration and failure of reinnervation. Heterologous fibrin biopolymer (HFB) is a promising sealant, but its long-term effects on the spinal cord and neuromuscular junctions (NMJs) are poorly understood. Objective: To investigate the effects of HFB, in combination with neurorrhaphy, on motor neuron survival, spinal cord glial reactivity, and NMJ ultrastructure restoration 120 days after sciatic nerve transection in rats. Methodology: Thirty-six male *Wistar* rats were divided into groups: Control (C), Denervated (D), Neurorrhaphy (N), and Neurorrhaphy + HFB (NB). After 120 days, glial reactivity (IBA-1 and GFAP) and sciatic nerve integrity (NF-200 - S100) were evaluated by immunohistochemistry. The ultrastructure of motor neurons and NMJs was examined by transmission electron microscopy. The study was approved by the local ethics committee (CEUA-FMB: 1402/2021). Results: Compared to the N group, the NB group demonstrated greater motor neuron survival and reinnervation. HFB normalized the glial response, with the NB group showing IBA-1 cell counts and a GFAP response closer to controls. Nerve integrity was higher in the NB group, with NF-200 and S100 patterns similar to those of controls. Ultrastructurally, the NMJ's in the NB group had more occupied synaptic grooves and preserved pre- and postsynaptic membranes, appearing healthier than the unoccupied grooves in the N group. Discussion: HFB helps create a better spinal cord environment, leading to superior reinnervation of the target muscle. This is supported by evidence showing healthier NMJ in the HFB group, confirming its role in restoring functional connections. Conclusion: HFB, as an adjunct to neurorrhaphy, significantly improves motor neuron survival, normalizes glial reactivity, and promotes superior reinnervation with the formation of healthier NMJs.
Keywords: neurorrhaphy; neuroregeneration; regenerative medicine; neuromuscular junction; heterologous fibrin biopolymer

AREA: Health Sciences
SUBAREA: Public health

DIGITAL EDUCATION AS A TRANSLATIONAL TOOL FOR THE PREVENTION OF ACCIDENTS INVOLVING VENOMOUS ANIMALS

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Accidents involving venomous animals are a recurring public health challenge in Brazil, especially in residential and rural areas. Lack of reliable information on species identification, biology, and first aid perpetuates myths, increasing the risk of accidents. Translational science is essential to turn scientific knowledge into accessible educational practices, preventing accidents and promoting community health. This project translates scientific discoveries on venomous animals into digital content, promoting environmental education and accident prevention. It connects basic research, translational science, and public health by providing clear, evidence-based guidance through digital platforms. CEVAP's Instagram was used as a dissemination tool, producing informative posts, short videos, templates, and interactive stories on accidents, prevention, species identification, and debunking myths. Content was based on literature review and expert curation, with platform analytics monitoring reach and engagement. The profile grew from ~1,700 to ~3,200 followers, with increases of 65.2% in activity, 57.1% in visits, 106.6% in external link clicks, and nearly 200% in business interactions. Recent posts exceeded 17,000 views, showing reach beyond the follower base. Interactive stories combined with posts and videos strengthened engagement and promoted safe practices. Digital media effectively democratizes information and reinforces translational science, guiding behavior change, reducing risks, and supporting preventive public health strategies. This project demonstrates the potential of digital environmental education to integrate basic research into everyday practices, expand scientific outreach, and contribute to policies that reduce accidents with venomous animals in Brazil. Therefore, we acknowledge and thank CNPq for granting the undergraduate research scholarship, which enabled the development of this work.
KEYWORDS: Venomous animals; Public health; Environmental education; Digital media; Accident prevention
FUNDING: PIBIC-SISPROPE, CNPq – Brazil.

GENOTOXIC AND MUTAGENIC EVALUATION OF ZEBRAFISH (*DANIO RERIO*) ADULTS CHRONICALLY EXPOSED TO THE HERBICIDE 2,4-D (DICHLOROPHENOXYACETIC ACID) AND ITS COMMERCIAL FORMULA DMA® 806 BR

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2,4-D pesticide is one of the most used in the world for controlling broad-leaved weeds in various crops. Despite that, it can affect the health of aquatic organisms even in low concentrations in the water. The objective of this study was to evaluate the genotoxic and mutagenic potential of 2,4-D acid in pure form (100%) and its commercial formula DMA® 806 BR on *Danio rerio* adults. The procedures were conducted in accordance with OECD Guideline No. 203 (2019) and the American Society for Testing and Materials (ASTM E729, 2014). A chronic exposure of 3 months was carried out with zebrafish adults, featuring a semi-static system (solutions were renewed each seven days). A total of 96 fish were used. Six fish were allocated to each glass aquaria/replicate, at a density of 1 animal per liter, and exposed to concentrations of 2,4-D and DMA 806 at 0.1, 5, and 30 µM. Controls included a Negative Control (without substance addition) and a Solvent Control (DMSO 0.01%). There were two replicates for each treatment. On the final day of the experiment, the fish were anesthetized and euthanized with benzocaine hydrochloride, for blood collection. Smears were done on glass slides and stained with acridine orange. To evaluate mutagenicity, micronuclei (MN) were identified based on the presence of extranuclear bodies with spherical or oval shapes. To evaluate genotoxicity, erythrocytes exhibiting nuclear abnormalities (NA) were evaluated. The relative frequencies of NA and MN were determined by dividing the number of abnormalities and micronuclei counted by the total number of erythrocytes examined (1000 nuclei). Statistical analysis was performed using the normality test, followed by analysis of variance and subsequently the Dunnet test. Regarding micronucleus test, there was no significant difference between the treatments and the control, resulting in a low possibility of these herbicides being mutagenic for zebrafish adults. The genotoxicity test resulted in a significant difference in concentrations of 0.1; 5 and 10 μM compared to the negative control, which results in the possibility of 2,4-D and DMA® 806 being genotoxic for zebrafish adults, in a long-term exposure at environmentally relevant concentrations.

KEYWORDS: pesticide; 2,4-D; zebrafish; abnormalities; micronucleus.
FUNDING: Coordination for the Improvement of Higher Education Personnel (CAPES), The São Paulo Research Foundation (FAPESP: 2020/07363-5; 2022/03045-4; 2018/00229-1), Center for Evaluation of Environmental Impact on Human Health (TOXICAM) and Institute of Biosciences of Botucatu (UNESP).
Ethical approval: Animal Ethics Committee (CEUA) nº. 1382/2021.

ANTIOXIDANT EFFECT OF BERGAMOT LEAF EXTRACT ON KIDNEY FUNCTION IN OBESE RATS

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Introduction: The chronic consumption of high sugar-fat diets (HSF) is one of the main contributors to the development of obesity in the 21st century. One of the consequences of obesity is oxidative stress (OS), a condition characterized by enhanced generation of reactive oxygen species (ROS) and reduced antioxidant defense. Increased OS contributes to the progression of kidney damage, often manifested by proteinuria. Natural compounds with antioxidant properties have been studied to enhance antioxidant capacity and protection against the ROS excess. Among them, *Citrus bergamia*, commonly known as bergamot, has emerged as a promising candidate. Extracts obtained from bergamot leaves have shown higher concentration of flavonoids compared to other parts of the plant, suggesting they may represent a promising treatment strategy. Objective: To investigate the antioxidant effect of bergamot leaf extract on kidney function in obese rats. Methodology: Male *Wistar* rats were randomized into two groups: Control (C; n = 10) and High sugar-fat diet (HSF; n = 20). After 20 weeks, obesity induction was confirmed by measuring body weight (g) and triglycerides levels (TG; mg/dL). The HSF group was isolated and redistributed into two groups (n=10): HSF and HSF supplemented with bergamot leaf extract (HSF+Lf). The supplementation of Lf (50mg/kg) was administered daily by gavage for 10 weeks. At the end of 30 weeks were analyzed: caloric intake (kcal/day), adiposity index (AI, %), the activity of the antioxidant enzymes superoxide dismutase (SOD, U/mg protein/minute) and catalase (CAT, pmol/g protein/minute), as well as proteinuria assessed by the protein/creatinine ratio (PROT/CREAT). Statistical analysis included Student's t-test ($\alpha = 0.05$). Results: At week 20, TG was significantly higher in HSF vs C ($p < 0.001$). After 10 weeks of treatment, no significant differences between HSF and HSF+Lf were found in caloric intake ($p = 0.921$) or AI ($p = 0.153$). However, significant increases were observed in antioxidant enzymes activity in the HSF+Lf group compared to the HSF group: SOD ($p = 0.033$) and CAT ($p = 0.049$). Furthermore, PROT/CREAT levels decreases in the HSF+Lf group compared to the HSF group ($p = 0.017$). Conclusion: Supplementation with bergamot leaf extract increased renal antioxidant enzyme activity and reduced proteinuria in obese rats. These results suggest that bergamot leaf extract exerts benefical effects against obesity-related oxidative stress in the kidney.

KEYWORDS: Nutraceuticals; Flavonoids; Antioxidant.
FUNDING: FAPESP (2025/01524-0).
Ethics Committee Approval: CEUA - 1337/2019.

PROTEOMIC AND FUNCTIONAL CHARACTERIZATION OF ANTIMICROBIAL PEPTIDES DERIVED FROM FISHERIES BYCATCH VIA ENZYMATIC HYDROLYSIS

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Introduction: The marine ecosystem is a prolific source of unique bioactive compounds with therapeutic potential. Over 41,000 molecules have been identified from marine organisms, some already FDA-approved. Fisheries bycatch, though ecologically problematic, represents underutilized biomass that, when ethically explored, may serve as a resource for biotechnological innovation, aligning with circular bioeconomy principles. Objective: This study characterized proteomic and functional profiles of hydrolysates from three bycatch species: the fishes *Paralonchurus brasiliensis* and *Micropogonias furnieri*, and the crab *Hepatus pudibundus*. We hypothesized that (1) enzymatic hydrolysis with Alcalase and Protamex would release diverse peptides, and (2) enzyme selection would shape functional outcomes, driving selective antimicrobial activity. Methodology: Muscle homogenates were hydrolyzed with Alcalase or Protamex. SDS-PAGE, RP-HPLC, MALDI-TOF, and LC-MS/MS were used for proteomic characterization. De novo sequencing and bioinformatics predicted bioactivities. Hydrolysates were tested in antimicrobial assays against resistant pathogens. Results: Both enzymes hydrolyzed muscle proteins extensively. RP-HPLC revealed complex peptide mixtures and enzyme-specific patterns. MALDI-TOF confirmed diversity, while LC-MS/MS identified thousands of peptide sequences. BioPep analysis predicted ACE inhibition, antioxidant, α-glucosidase and renin inhibition, and bacterial permease ligands. Functionally, Protamex hydrolysate of *P. brasiliensis* showed strong antifungal activity against *Candida albicans* (81%), while Alcalase hydrolysate had moderate activity against *Staphylococcus aureus* (29%). Discussion: The selective anti-*Candida* activity underscores the role of enzyme choice and reveals therapeutic potential in fisheries bycatch. These results expand evidence of marine-derived antimicrobial peptides, including activity against resistant pathogens. Predicted multifunctional properties suggest synergistic benefits, supporting applications in pharmaceuticals and nutraceuticals. Conclusion: Valorizing fisheries waste transforms an ecological problem into high-value bioactive compounds. Protamex–*P. brasiliensis* hydrolysate emerges as a promising source of anti-*Candida* peptides.
KEYWORDS: Bycatch; marine peptides; antimicrobial peptides; proteomics; *Candida albicans*.
FUNDING: CAPES.

TRANSLATIONAL EFFECTS OF BERGAMOT BY-PRODUCT IN AN EXPERIMENTAL OBESITY MODEL: INFLAMMATORY, OXIDATIVE AND BODY COMPOSITION MODULATION

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Introduction: Obesity is characterized by alterations in body composition, low-grade chronic inflammation and increased oxidative stress in adipose tissue, which plays a central role in the development of diseases. In this context, bioactive compounds have emerged as potential translational tools, capable of accelerating the application of experimental findings into clinical and therapeutic practices. Among them, the bergamot by-product *(Citrus bergamia)* has been investigated for its antioxidant and anti-inflammatory properties, suggesting a promising effect in modulating the inflammatory microenvironment of adipose tissue and improving body composition in obesity. Objective: To evaluate the effects of bergamot by-product (BB) on body composition, inflammation, and oxidative stress in the adipose tissue of obese rats. Methodology: *Wistar* rats (n=24) were distributed into two groups: Control (C) and Obese (OB) to induce obesity by hypercaloric diet + 25% sucrose in drinking water for 20 weeks. After this period, they were redistributed into four groups (n=6): C, C+BB, OB, and OB+BB for 10 weeks. BB was administered by gavage (250mg/Kg). Evaluated parameters included adiposity index, quadriceps muscle mass, body weight, and adipose tissue inflammatory markers (Interleukin-6 (IL-6), Interleukin-10 (IL-10), and myeloperoxidase (MPO)) and oxidative stress markers (Malondialdehyde (MDA), and carbonylated proteins (CBO)). Statistical analyses were performed using two-way ANOVA followed by Tukey's post-hoc test ($p<0.05$). CEUA protocol nº 1470/2025. Results: The OB group exhibited increased adiposity index, IL-6, MPO, MDA, and CBO levels, and decreased muscle mass and IL-10 compared to the C group. The OB+BB group showed a lower level of adiposity index, IL-6, MPO, MDA, CBO and higher quadriceps mass and IL-10 levels relative to OB, without changes in body weight. Discussion: BB attenuated inflammation and oxidative stress and promoted favorable remodeling of body composition, preserving muscle mass and reducing adiposity under obese conditions. These findings highlight its translational potential as an adjuvant intervention in obesity management and in mitigating its adverse effects on body composition. Conclusion: Bergamot by-product exerted anti-inflammatory, antioxidant, and body composition-modulating effects in obese rats, representing a promising strategy for future clinical and therapeutic applications.

KEYWORDS: obesity; body composition; inflammation; oxidative stress; translational science.

FUNDING: FAPESP (IC Grant nº 2023/09275-4; Regular Grant nº 2021/13050-2).

ADVANCING DRUG SAFETY ASSESSMENT THROUGH A BIOPRINTED HEPG2 HYDROGEL MODEL

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Three-dimensional (3D) bioprinting has emerged as an alternative technology that enables the controlled, scalable, and personalized assembly of cells, biomaterials, and biomolecules. In toxicology, liver cell–based bioprinted constructs provide physiologically relevant platforms capable of reproducing key metabolic functions of the liver, allowing more accurate assessment of drug-induced hepatotoxicity, xenobiotic metabolism, and chemical safety profiles. The objective of this study was to develop a HepG2-laden hydrogel (20% Pluronic F127/2% sodium alginate) bioprinted into liver-mimetic constructs. After that, the cytotoxic potential of the emerging contaminants triclopyr and decamethylcyclopentasiloxane (D5) was identified at concentrations of 5, 50, and 500 μM for 24 and 48 hours. The constructs were evaluated by Live/Dead staining and metabolic activity assays. On the day-7, the culture medium was exposed to triclopyr or D5 standard solutions (5–500 μM, 0.1% DMSO), with 0.1% DMSO alone as the negative control. After exposure, cell death, mitochondrial membrane potential, and 3D construct histology were analyzed. During the 14-day culture period, the hydrogel maintained high cell viability and proliferation, confirmed by fluorescence microscopy, histological analyses using H&E, PAS, and Masson's trichrome staining, as well as metabolic assays. Assessments indicated no notable toxicity within the 3D bioprinted hydrogel, either in terms of cell viability or mitochondrial membrane potential, in contrast to findings previously described for 2D cultures for triclopyr. The reduced sensitivity exhibited by the 3D model is presumably associated with its elevated structural complexity, more realistic cell–cell interactions, and modified receptor distribution. In conclusion, the 3D bioprinted liver model is a physiologically relevant system for assessing hepatotoxicity and a promising substitute for conventional 2D cultures in toxicological research. It supports the principles of new approach methodologies (NAMs) aimed at translational science to reduce reliance on animal testing while paving the way for broader application in drug safety assessment.

KEYWORDS: HepG2 cells; hepatotoxicity; cytotoxicity; mitochondrial dysfunction
FUNDING: This research was supported by the São Paulo Research Foundation (FAPESP) [Grant No. 2020/11128-1 e 2022/03045-4]; the Coordination of Superior Level Staff Improvement - Institutional Internationalization Program (CAPES - PrInt) [Grant No. 88887.696580/2022-00]; and by the Center for Evaluation of Environmental Impact on Human Health (TOXICAM), UNESP, Brazil.

OUTER MEMBRANE VESICLES OF MENINGOCOCCI BOOST IMMUNOGENICITY OF RECOMBINANT SARS-COV-2 PROTEIN AND OFFER TRANSFERABLE IMMUNITY FROM MOTHERS TO OFFSPRING WITH HIGH AVIDITY AND NEUTRALIZING ANTIBODIES

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Maternal immunization protect mothers and newborns. Meningococcal disease, caused by meningococci, mainly affects children up to two years of age, and important complications were observed following COVID-19 in this group. We proposed a preparation containing outer membrane vesicles (OMVs) from meningococci C:2a:P1.5 strain, associated with the receptor-binding domain (RBD) of SARS-CoV-2 (Wuhan) and alum (AH), to enhance immunogenicity and support maternal–fetal transference of IgG. Three groups of BALB/c ($H2^d$) mice were immunized with different RBD doses: A) 0.5μg, B) 1.0μg, or C) 1.5μg, each combined with 0.5μg OMV+0.1mM AH. The control group (D) was naïve. Mice were mated 30 days after the last dose, blood was collected from the offspring and from their mothers 21 days after birth (CEUA/IAL 01/2023). IgG, IgG1, IgG2a, IgG2b, and IgG3 were measured by ELISA; antibody functionality was evaluated by avidity and a cytopathic effect-based virus neutralization test with SARS-CoV-2. The RBD-specific response showed that all groups had higher IgG levels than the control ($p<0.001$), with a predominance of IgG1 ($p<0.001$). Groups B and C exhibited increased levels of IgG2a and IgG2b ($p<0.05$). The mean avidity (%) and neutralizing titers were, respectively: A) 46.25% and 1/80; B) 85.41% and 1/320; C) 71.05% and 1/320. Offspring from all groups had higher IgG than the control ($p<0.001$), with the following avidities (%) and neutralizing titers: A) 93.68 and 1/160; B) 96.6 and 1/640; C) 90.3 and 1/640. The respective results of their mothers were: A) 95.35 and 1/160; B) 99.04 and 1/1024, C) 94.33 and 1/320. The OMV-specific response showed enhanced IgG for all groups compared with the control ($p<0.001$); IgG1 and IgG2b were the main isotypes ($p<0.001$). The mean avidity (%) was: A) 81.98, B) 80.62, and C) 74.95. Offspring from all three groups presented higher IgG levels than the control ($p<0.001$), with mean avidities (%) of: A) 79.56; B) 95.64; and C) 84.4. These results suggest that OMVs can be used to enhance the immunogenicity of recombinant proteins while conferring anti-meningococci response, in both mothers and their offspring. Serum bactericidal assay would be required to confirm protection from meningococci. Thus, OMVs are feasible to produce and have previously been approved for human use, providing a precedent that facilitates the translation of these antigenic preparations into clinical trials.

KEYWORDS: COVID-19; Meningococcal disease; Outer membrane vesicles; maternal-fetal immunization.

FUNDING: FAPESP (18/04202-0, 20/08943-5, 21/11936-3, 21/05661-1, 22/05566-1, 23/07287-5), CNPq (305301/2022-5), CAPES (finance code 001).

RIBOTYPE PROFILE OF CLOSTRIDIOIDES DIFFICILE IDENTIFIED IN FOALS

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Introduction: Clostridioides difficile is important agent of gastrointestinal disorders in animals and humans. Certain ribotypes (RTs) show higher incidence in some species; however, some RTs are detected across multiple hosts. In this context, horses are important epidemiological agents, as they are not only susceptible to this pathogen but also maintain close contact with humans, especially workers directly involved in their handling. Materail and Methods: The present study aimed to describe the RTs of C. difficile identified in foals. The project was approved by CEUA/FMVZ/Unesp (protocols 95/2020, 59/2024, and 142/2024). A total of 600 fecal samples from foals were analyzed. Samples were initially inoculated into non-selective fructose broth enriched with sodium taurocholate and incubated at 37 °C for seven days. Subsequently, an alcohol shock was performed, followed by seeding on CDMN agar and incubation at 37 °C for five days under anaerobic conditions. Colonies with suggestive morphology were subjected to multiplex PCR (16S rDNA, tcdA, tcdB, cdtA/B), followed by amplification of the intergenic region (23S–16S) and fragment analysis by capillary electrophoresis. The resulting data were submitted to WEBRIBO for RT determination. Results: C. difficile was detected in 18% (108/600) of the samples, with 63% (58/108) being A–B– CDT–, 10% (11/108) A+B+ CDT+, and 31% (34/108) A+B+ CDT+. The identified RTs were: 010 (39.8%), 126 (25.9%), 009 (11.8%), 046 (5.5%), AI-60 (3.7%), 066/2 and 557 (2% each), 754 and 078/4 (0.9% each), while 8.3% were classified as novel RTs. Discussion:The predominant RTs observed (010 and 126) are also frequently reported in humans. Although RT 010 does not carry toxin-encoding genes, it has been described as causing enteric alterations in immunosuppressed patients in intensive care units. RT 126, considered hypervirulent, is associated with colitis in humans, swine, and horses. These findings highlight the importance of horses as potential disseminators of C. difficile, both through the risk of colonization and the spread of resistance genes and virulence factors. They also emphasize the role of horses within a One Health framework, as they share environments with multiple animal species and maintain close contact with humans, potentially serving as a source of exposure to different C. difficile lineages. Conclusion: The predominante RTs identified in foals were 010 and 126.

KEYWORDS: Horses, RT126, One Health
FUNDING: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (2024/00964-4; 2024/01073-6 and 2024/01072-0).

AREA: Health Sciences
SUBAREA: Public Health

THE IMPACT OF EXPANDED SCREENING AND TREATMENT ON THE INCIDENCE OF SYMPTOMATIC CHLAMYDIA AND GONORRHEA IN MEN ON HIV PREEXPOSURE PROPHYLAXIS

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Introduction: Early diagnosis and treatment of sexually transmitted infections (STIs) caused by *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) in men are key to preventing complications such as infertility. Although these infections are often asymptomatic, the impact of early diagnosis and treatment on transmission remains uncertain, especially among HIV preexposure prophylaxis (PrEP) users and men who have sex with men (MSM). Currently, most PrEP clinics only conduct annual urine-based screening. Objective: To assess the impact of early diagnosis and treatment of CT and NG in asymptomatic MSM on PrEP, with a focus on changes in the incidence of symptomatic infections. Materials and Methods: This cohort study included 275 MSM followed at a PrEP clinic (SAEI-DAM, Unesp Botucatu). Beginning in 2024, an intervention was implemented to treat all asymptomatic individuals identified through recurrent screening of urine, anal, and oral samples every four months. Test results (positivity for CT or NG in symptomatic and asymptomatic patients) were retrieved from electronic medical records and divided into two periods: "pre-intervention" (2022–2023) and "post-intervention" (2024–2025). Descriptive analysis and chi-square trend tests were used to assess changes in CT and NG proportions ($p<0.05$). CAAE: 78279824.2.0000.5411. Results: After the intervention, 31 asymptomatic CT and 20 NG cases were detected, whereas no asymptomatic cases were found prior to the expanded, recurrent screening. For symptomatic cases, the incidence of CT decreased significantly from 19 (6.9%) pre-intervention to 7 (2.5%) post-intervention ($p=0.016$). NG showed 10 (3.6%) symptomatic cases before and 6 (2.2%) after ($p=0.310$). Discussion: The rise in asymptomatic CT and NG cases reflects the expanded and periodic screening strategy that included multiple anatomical sites every four months. Although the intervention did not significantly reduce symptomatic NG cases, it markedly decreased symptomatic CT cases, showing its effectiveness in interrupting transmission and reducing complications. Conclusion: Expanded, routine extragenital screening was effective in reducing symptomatic CT cases, underscoring the positive impact of early diagnosis and treatment. However, the persistence of NG highlights the need for complementary strategies, such as antimicrobial resistance surveillance and partner management, to effectively control this infection.

KEYWORDS: STIs. Diagnosis. Symptoms.
FUNDING: Coordination for the Improvement of Higher Education Personnel (CAPES)

USE OF PHENOLIC ACID-DERIVED BIOMODIFIERS: ANTIMICROBIAL AND ANTI-BIOFILM EFFECTS ON DENTIN BOND STRENGTH

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Adhesive systems provide the bonding interface between the dental substrate and resinous compounds. This interface can be degraded by the activity of Matrix Metalloproteinases (MMPs). The use of natural biomodifiers such as phenolic acids (PAs), cinnamic acid (CA), and p-coumaric acid (p-CA) can reduce MMP-mediated degradation due to the antimicrobial, antioxidant, and anti-inflammatory properties of PAs. The aim of this study was to evaluate the antimicrobial effect of PA derivatives against *Streptococcus mutans* and *Candida albicans* and to assess their effect on dual-species biofilm through colony-forming unit (CFU) counts. Thirty-two human molars were treated with the compounds and sectioned along their longitudinal axis, resulting in four experimental groups: control, treatment with LA11 test solution (LA11), treatment with C-Methyl test solution (C-Methyl), and treatment with 2% chlorhexidine solution (CHX).The antimicrobial activity of PA derivatives was determined by minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) assays against the dual-species biofilm. Subsequently, the anti-biofilm effect was assessed by CFU counts. Data were analyzed using one-way ANOVA followed by Tukey-Kramer test ($\alpha = 0.05$).All tested phenolic derivatives exhibited inhibitory microbial effects, with MIC values ranging from 15.6 to 1000 μg/mL. Both compounds demonstrated microbicidal activity, particularly LA11, which achieved 100% reduction of the dual-species biofilm. It is concluded that the use of PA derivatives exhibited antimicrobial and anti-biofilm effects on the dual-species biofilm.

KEYWORDS: Antimicrobial activity; Biomaterials; Phenolic compounds
Ethics Committee: Approved by the Research Ethics Committee of the School of Dentistry of Araçatuba – UNESP (FOA), Protocol No. 108138/2022.

MAXIMIZING SCREENING, MINIMIZING COSTS: SELF-SAMPLING AND POOLING FOR CHLAMYDIA AND GONORRHEA IN MEN USING PREP

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Introduction: The global burden of sexually transmitted infections (STIs) such as chlamydia (CT) and gonorrhea (NG) is a growing concern, especially among men who have sex with men (MSM) on HIV pre-exposure prophylaxis (PrEP). High asymptomatic prevalence in this population highlights the need for regular, expanded screening. Urine-only testing is often insufficient, as extragenital sites are frequently implicated due to common sexual practices. Objective: This study aimed to determine the incidence of asymptomatic CT and NG in MSM on PrEP, compare the diagnostic sensitivity of urine, anal, and oropharyngeal samples, and evaluate the acceptability of self-sampling and pooled molecular testing as innovative screening strategies. Methods and Methods: We conducted a prospective cohort study of 111 MSM on PrEP, who performed self-sampling of urine, anal, and oropharyngeal specimens. Real-time PCR was used for detection on individual and pooled samples. A questionnaire assessed self-sampling acceptability. Statistical analyses included chi-square and diagnostic performance tests. CAAE: 78279824.2.0000.5411. Results: CT positivity was highest in anal samples (13%, $p<0.0001$), followed by oropharyngeal (3%) and urine (2%). For NG, positivity was highest in oropharyngeal and anal samples (6%), significantly higher than in urine (1%, $p=0.0022$). Pooled testing showed high concordance with individual samples, with sensitivities ranging from 80–100% for CT and 60–100% for NG. Self-sampling and regular adherence were highly accepted (98.1%). Discussion: Our findings highlight the high rates of asymptomatic STIs in MSM on PrEP, predominantly at extragenital sites. The elevated positivity of anal CT and oropharyngeal NG indicates that urine-only testing significantly underestimates true incidence. The high acceptability of self-sampling, combined with sexual risk practices, underscores the need for more accessible and comprehensive screening. Self-sampling with molecular testing is a viable and effective approach for early detection and interruption of transmission. Additionally, pooled testing may optimize costs, making expanded testing feasible in public health services. Conclusion: Self-sampling and pooled testing are viable, sensitive, and cost-effective strategies for STI screening among MSM on PrEP. These innovative approaches reinforce the need for broader extragenital testing to improve detection and ultimately reduce the burden of STIs in this population.

KEYWORDS: Chlamydia; Gonorrhea; Asymptomatic; Self-sampling; Pooling.
FUNDING: FAPESP, CAPES.

AREA: Agricultural sciences and engineering
SUBAREA: Veterinary Medicine

EVALUATION OF CANDIDATE GENES ASSOCIATED WITH CONGENITAL MICROPHTHALMIA IN BRAZILIAN WARMBLOOD HORSES

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Abstract: The ocular development of vertebrate animals is a complex process regulated by several genes, whose alterations may lead to anomalies such as microphthalmia, anophthalmia, and coloboma (MAC). These conditions have a strong genetic basis in humans, with more than 90 genes associated. In horses, reports are scarce, with isolated descriptions of unknown causes. The case of a Brazilian Sport Horse filly presenting with congenital bilateral microphthalmia prompted the investigation of pathogenic variants. Therefore, the aim of this study is to investigate variants in candidate genes, which are associated with ocular malformations in humans and other animal species, in Brazilian Warmblood horses. To date, PCRs of the coding sequences of the OTX2, SOX2, PITX3, RAX, and RBP4 genes have been standardized using DNA from a control (healthy) animal and from the affected filly. The PCR products were purified and subsequently sequenced by the Sanger direct method, and the sequences obtained from the affected filly were compared with those deposited in databases and with the sequence from the healthy animal. To date, no pathogenic variants have been detected in the analyzed coding regions. However, by leveraging established knowledge in human genetics, it was possible to focus the investigation on genes previously associated with ocular malformations. Although a negative result was obtained, this allows for the exclusion of an association between these genes and the observed phenotype, suggesting that other genes involved in ocular development may be implicated in this congenital alteration. Finally, the study will continue with the analysis of new candidate genes related to microphthalmia/anophthalmia in humans and other animal species.
KEYWORDS: genetic variant, ocular anomalies, ophthalmology
FUNDING: Recipient of a CAPES PhD Scholarship and FAPESP process 25/09744-0
Ethics: Approved by CEUA/FMVZ 217/2021

AREA: Agricultural scinces and engineering
SUBAREA: Veterinary Medicine

DETERMINATION OF HEAVY METALS IN BLOOD OF BLACK VULTURES (CORAGYPS ATRATUS) IN SÃO PAULO STATE, BRAZIL

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Introduction: Vultures are important scavengers, living widespread in the world. These are susceptible to greater exposure to chemicals through bioaccumulation and biomagnification. This population has been declining and the mortality rates due to heavy metal poisoning are one of the causes. Due to their position in the food chain, vultures serve as sensitive indicators of environmental contamination. Objectives: To determine concentration of heavy metals in the blood of free-living *Coragyps atratus* (Black Vulture). Methodology: This study was approved by the Ethics Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science (074/2024) and by the Ministry of the Environment through the Biodiversity Authorization and Information System (SISBIO) (93356-1). Black vultures (*C. atratus*) (n=10) were captured using a walk-in trap at the School of Veterinary Medicine and Animal Science of UNESP. Following restraint, they underwent clinical evaluation by veterinarian. Blood samples were collected by puncturing the radial or metatarsal vein. Heavy metals (arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg) and lead (Pb) were analyzed using atomic absorption spectrophotometry. Results: The mean dosages (and standard deviation) of As was 248.0±36.33 μg/dL, Cd 4.69±0.31 μg/dL, Cu 23.4±3.09 μg/dL, Hg 56.62±21.5 μg/dL, and Pb 17.5±3.44 μg/dL. Concentrations of As were not detected in samples from five *C. atratus*, while Hg was not detected in two animals. Discussion: There are no established thresholds for heavy metal concentrations in *C. atratus*. Therefore, measurements are compared with those reported for other vulture species. Regarding Pb, concentrations below 20 μg/dL are considered indicative of normal exposure. Cd concentrations above 0.05 μg/dL are associated with oxidative alterations in birds, as are Hg concentrations above 3 μg/dL and Pb concentrations above 15 μg/dL. Comparisons with other studies indicate that the vultures analyzed present Cu levels within the normal range. On the other hand, the levels of As, Cd, and Hg observed in the evaluated animals appear to be elevated, suggesting that these vultures are possibly exposed to these molecules. Conclusions: The observation of elevated values reinforces the need for further studies to establish reference values for this species, as well as to identify risk factors associated with elevated concentrations.

KEYWORDS: Environmental contamination; mercury (Hg); lead (Pb), copper (Cu);
FUNDING: The authors are thankful for the support provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Finance Code 001.

AREA: Agricultural sciences and engineering
SUBAREA: Veterinary Medicine

ETIOLOGICAL AGENTS OF DIARRHEA IN FOALS: IMPLICATIONS IN THE CONTEXT OF ONE HEALTH

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Abstract: Diarrhea is one of the main causes of mortality in foals, with a significant impact on horse breeding. Infectious causes, such as bacterial, viral, and parasitic agents, are the most common. Identifying these agents is extremely important for an effective therapeutic approach in these animals, in addition to its relevance within the context of one-health, given the presence of microorganisms with zoonotic characteristics. The objective of this retrospective study was to identify the main etiological agents associated with diarrhea in foals treated at the UNESP/FMVZ large animal clinic in Botucatu, São Paulo, and to discuss their implications in the context of One Health. Thirty-three cases of foals up to one year of age with clinical signs of diarrhea were evaluated. A total of 30 microbiological cultures were performed, with *Escherichia coli* (74%, 25/30) and *Salmonella spp*. (41%, 14/30) being the most isolated agents. qPCR was performed in eight cases (24%, 8/33), where the agents *Clostridioides difficile* (38%; 5/8 cases), *Clostridioides difficile* toxin B (25%, 2/8 cases), and *Clostridium perfringens*, Rotavirus, and *Giardia spp*. (12%, 1/8 cases) were detected. Microbiological stool culture and qPCR are widely used in routine etiological diagnosis of neonatal diarrhea.The identification of agents such as *Escherichia coli*, *Salmonella spp.*, *Clostridium spp.*, *Giardia spp.*, and Rotavirus reinforces the multifactorial nature of diarrhea in foals and highlights its importance in the context of one health, since these microorganisms are among the main causes of severe diarrhea in humans. Thus, these results highlight the importance of integration between veterinary and human medicine and contribute to the improvement and implementation of biosafety and epidemiological surveillance protocols, which are fundamental for minimizing the risk of disease transmission and promoting animal, human, and environmental health.
KEYWORDS: foal diarrhea; infectious agents; one health; epidemiology.
FUNDING: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (2024/21908-5).

OUTCOMES OF A TELEMEDICINE SYSTEM FOR COVID-19 IN BRAZIL: A PROSPECTIVE COHORT STUDY

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The sudden emergence of SARS-CoV-2 in late 2019/early 2020 severely strained global health systems, exposing weaknesses in surveillance, especially in data reporting and contact tracing. ICU occupancy peaked in 2020, reducing non-COVID care. In this context, telemedicine became a strategic solution. This study evaluated E-care Sentinel, a telemedicine system implemented at Sao Paulo State University (Unesp) from June 2021 to June 2024, aimed at reducing hospital burden, enabling early case detection and isolation, monitoring deterioration, and ensuring continuous support. A prospective cohort included 6,129 adults with COVID-19-like symptoms, among workers, students, and families across 34 campuses in 24 cities. Physicians attended 80% (4,903/6,129), prescribed medications to 28% (1,411/5,041), and issued work leave to 43% (2,635/6,129). Testing was recommended for 24%, with positivity above 81%. Only 66 patients (1.2%) required in-person care, and no COVID-19-related deaths occurred. Satisfaction was remarkably high, with 96% (5,584/6,129) rating the service positively. By serving over 6,000 patients remotely, the system minimized hospital overload, reduced face-to-face visits, and mitigated transmission risks. The results highlight telemedicine's effectiveness in pandemic response and reveal an unmet demand for virtual care. Findings provide robust evidence for expanding such models, emphasizing the role of multidisciplinary approaches in preventing comorbidities. The project was supported by Unesp and the VUNESP Foundation and approved by the Research Ethics Committee of Botucatu Medical School (protocol 4,002,318).

KEYWORDS: Telehealth; Teleconsultation; Remote consultation; Public health; COVID-19.
FUNDING: Unesp and Vunesp Foundation.

NEGLECTED SPACES, EMERGING THREATS: *DIROFILARIA IMMITIS* IN THE PERIPHERAL COMMUNITIES OF RIO DE JANEIRO, BRAZIL

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Dirofilariosis, caused by the mosquito-borne parasite *Dirofilaria immitis*, is an emerging public health concern in Brazil, with an increasing prevalence in regions historically considered non-endemic. While various studies have investigated potential risk factors influencing parasite transmission, findings have often been inconsistent, highlighting the complexity of its epidemiology. Environmental and socioeconomic conditions are likely to play a pivotal role in shaping disease distribution. This study aimed to elucidate key environmental risk factors associated with *D. immitis* transmission by conducting a cross-sectional analysis of 497 dogs distinct urban and peripheral landscapes in Rio de Janeiro, Brazil. A multi-diagnostic approach integrating parasitological, serological, and molecular methods was employed to provide a more robust and comprehensive assessment. The prevalence of *D. immitis* was significantly higher in peripheral, low-income areas (14.47%) compared to the urban area of Rio de Janeiro (6.17%) ($p = 0.003$, adjusted OR = 0.28 (95% confidence interval: 0.13–0.64)). Dogs residing in socioeconomically disadvantaged regions, characterized by inadequate sanitation, ineffective waste management, and environmental degradation, exhibited a markedly increased risk of infection. Additionally, cost-constraints and limited access to veterinary services in peripheral areas lead to low adherence to preventive measures. These findings underscore the significant impact of socioeconomic inequities on the epidemiology of dirofilariosis. Poor infrastructure, environmental degradation, and restricted access to veterinary care contribute to the sustained transmission of *D. immitis* in marginalized communities. Addressing these disparities through strengthened public health policies, targeted vector control measures, and enhanced community education is imperative for mitigating the risk of infection in both animals and humans.

KEYWORDS: Dirofilariosis, Mosquito-borne infection, Environmental degradation, One Health, Economic disparity.

FUNDING: Chocobar M.L.E received a Ph.D. grant (88887.726177/2022-00) and a training grant (88887.936596/2024-00) from CAPES (Coordination for the Improvement of Higher Education Personnel).

AREA: 1 Biological Sciences
SUBAREA: 1 General Biology

IN VITRO ANALYSIS OF THE MECHANISMS OF TOXICITY INDUCED BY THE HERBICIDE 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) IN THE C2C12 CELL LINE

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To ensure the protection of human health and the environment, it is essential to adequately verify the toxicological potential of chemicals to which humans are exposed in their daily lives: pesticides, pharmaceuticals, industrial chemicals, among other substances, whether through production, consumption, presence in the environment, or improper disposal. This study, which is in line with the global trend toward using alternative methods for assessing the risk of chemical compounds, aimed to evaluate the toxicity of a representative of emerging environmental contaminants of concern, the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and C2C12 cell model. The concentrations used in this study were based on concentrations already found in the environment and in studies in the literature with this compound, namely 0.1, 4, 10, 100, and 200 µmol/L, as well as the negative control (NC) and positive control (PC). The results obtained were statistically compared using the One-way ANOVA method (Graphpad Prism 9.0), comparing the treatments with different concentrations of the compound with its negative control (NC), followed by the Dunnet test. For the analysis of metabolic capacity, MTT experiments were used, in which the cell metabolizes it, generating formazan salt, and SRB, in which sulphorhodamine-β binds to cellular proteins, making it possible to evaluate their quantity and, therefore, their proliferation. The mitochondrial membrane potential assay uses the TMRM dye, which accumulates in healthy mitochondria and emits a fluorescence signal. No effect was observed in the evaluation of the cell's metabolic capacity. However, in the evaluation of cell proliferation at 48 hours, a significant reduction was observed in all concentrations except 5 µM. In the evaluation of mitochondrial membrane potential dissipation at 48 hours, there was an increase at all concentrations. Therefore, a possible alteration in the mitochondrial pathway in energy generation may be related, requiring further experiments to corroborate this analysis.

KEYWORDS: herbicide; toxicity; 2,4-dichlorophenoxyacetic acid; C2C12
FUNDING: FAPESP

INVESTIGATION OF THE C.102C>A PATHOGENIC VARIANT IN THE GBE1 GENE IN A BULL-CATCHING QUARTER HORSE IN BRAZIL ASSOCIATED WITH GLYCOGEN BRANCHING ENZYME DEFICIENCY: A POSSIBLE DISEASE MODEL FOR HUMANS

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Some hereditary diseases in horses could be relevant as experimental models for advancing the understanding of human diseases as well. Quarter Horses (QH) are widely recognized for their use in various equestrian sports, and several scientific studies have been conducted to characterize their genetic and performance traits, aiming to optimize their use. Bull-catching (vaquejada) is an important equestrian sport in Brazil, accounting for approximately 13% of the sport horse population in the country, particularly in the Northeast region. Studies on genetic disorders in QH are highly relevant within equine medicine and may be approached from a translational perspective, paralleling methodologies used in human medicine, such as Glycogen Branching Enzyme Deficiency (GBED) in horses and Glycogen Storage Disease Type IV (Andersen's disease) in humans. GBED is a fatal autosomal recessive disorder affecting QH and related breeds. It is caused by a nonsense c.102C>A pathogenic variant in the GBE1 gene, which disrupts glycogen synthesis and results in multisystemic energy failure, leading to abortion or early death of affected neonatal foals. Similarly, in Andersen's disease, a GBE1 variant is also responsible for this severe pediatric disorder. In the present study, Sanger sequencing was used to genotype the GBED variant in a population of 94 clinically healthy bull-catching QH from Northeastern Brazil. About 5.3% (5/94) of the bull-catching Quarter Horses (QH) were heterozygous for the pathogenic variant. When compared with previous studies, the frequency of this pathogenic variant was lower in cutting (19.75%) and reining (10% [16/160]) QHs, but higher than in barrel racing (5%), halter (3%), and racing horses (0%). In conclusion, the GBED pathogenic variant is present in bull-catching Quarter Horses (QH) and may impact breeding programs, as well as represent an economic burden due to mortality associated with GBED. Since GSD IV is a rare condition, with an estimated prevalence of 1 in 600,000 to 800,000 individuals globally, studies using GBED-affected foals could serve as a natural model for GSD IV, providing opportunities to advance the understanding of glycogen metabolism disorders and to improve diagnostic tools, preventive strategies, and clinical management for affected individuals.

KEYWORDS: GBED, Quarter Horse, allele frequency, glycogen storage disease
FUNDING: FAPESP (2024/01268-1)
Institutional Animal Care and Use Committee (215/2024- CEUA - UNESP)

USE OF HOLTER MONITORING TO EVALUATE HEART RATE VARIABILITY IN DOGS WITH PARVOVIRUS

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The canine Parvovirosis is an infectious disease caused by the type 2 (CPV-2) parvovirus, with two main clinical manifestations: enteric and myocardial. It is known that myocarditis can lead to changes in the electrocardiogram, such as second and third-degree atrioventricular block (AVB) and ventricular arrhythmias. The definitive diagnosis is made through histopathological examination of the myocardium, which is difficult to perform in routine clinical practice. The Holter Monitor is a device that enables continuous monitoring of cardiac electrical activity for up to 24 hours, allowing for greater sensitivity in detecting arrhythmias and assessing heart rate variability (HRV). Given the importance of cardiological evaluation in the prognosis of diseases that can affect cardiac function, the present study aimed to evaluate heart rate variability in the time domain in dogs infected with canine parvovírus in natural setting. Young canines infected with CPV-2 (GP) (n=10) and healthy young canines (GC) were evaluated. The animals underwent electrocardiographic evaluation using a 3-channel Holter Monitor for 3 hours. The following heart rate variability parameters were evaluated: SDNN (Standard Deviation of NN intervals), SDANN (Standard Deviation of the Averages of NN intervals in 5-minute segments), SDNNi (Mean of the Standard Deviations of NN intervals for each 5-minute segment), rMSSD (Root Mean Square of Successive Differences) and pNN50 (Percentage of successive NN intervals that differ by more than 50 ms). The results obtained showed statistically significant differences; in the parvovirus group, the mean values obtained were SDNN = 42.7 ± 26.3 (p=0.09), SDANN = 24.6 ± 14.6 (p=0.0012), SDNNi = 31.7 ± 21.2 (p=0.0012), rMSSD = 33.5 ± 22.9 (p=0.0012) and pNN50 = 6.2 ± 8.5 (p=0.0007). In the control group, SDNN = 132.3 ± 66.7, SDANN = 73.2 ± 37.3, SDNNi = 106.9 ± 58.1, rMSSD = 133.6 ± 79.3, and pNN50 = 34.1 ± 19.8. The reduction in HRV in sick animals suggests significant impairment of cardiovascular autonomic modulation in dogs infected with CPV-2 and indicates that the body is under significant stress, with sympathetic activation and vagal suppression, which can be used as an early indirect marker of systemic decompensation even before structural or functional cardiac changes become evident. These results reinforce the potential of HRV analysis as a complementary tool in clinical monitoring and prognosis of dogs affected by severe infectious diseases.

KEYWORDS: parvovirosis; myocarditis; Holter; arrhythmia; heart rate variability
FUNDING: This study was funded by the National Council for Scientific and Technological Development (CNPq), process number: 404212/2021-2.

CYTOTOXICITY ASSAY OF NISIN: A POTENTIAL TOPICAL THERAPY FOR CANINE PYODERMA

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Staphylococcus pseudintermedius is frequently isolated in the veterinary clinical routine of companion animals and represents an important cause of antimicrobial use. *S. pseudintermedius* is part of the natural skin microbiota of small animals; however, under dysbiosis conditions, this opportunistic agent proliferates, leading to the development of canine pyoderma. Natural antimicrobials have been studied as potential alternatives to conventional drugs due to their antibacterial, antifungal, and antiviral properties. Nisin, a bacteriocin produced by *Lactococcus lactis*, exhibits both antibacterial and anti-inflammatory activity. The increasing concern regarding antimicrobial resistance highlights the need for alternative therapeutic strategies. Thus, the aim of this study was to evaluate the cytotoxicity of nisin through the Neutral Red Uptake (NRU) assay, using BALB/c 3T3 clone A31 cells (murine fibroblast). The study was approved by the Ethics Committee on Animal Use (CEUA) of FMVZ-UNESP, Botucatu/SP (protocol nº 000.319). The minimum inhibitory concentration (MIC) of nisin against methicillin-resistant *S. pseudintermedius* (MRSP) was evaluated using the broth microdilution assay. For NRU assay, cells were exposed to eight concentrations of nisin (0.118 to 25.00 mg/mL) for 48 hours, and cell viability was assessed by the ability of viable cells to absorb Neutral Red dye. The results were used to determine the mean inhibitory concentration (IC50) of nisin and to predict *in vitro* acute oral toxicity. The MIC of nisin against MRSP isolate was 6.25 µg/mL and the IC50 value obtained was 5.8 mg/mL. Based on this value, the LD50 (lethal dose for 50% of individuals) was estimated at 2654.58 mg/kg, which may serve as a reference dose for future animal toxicity studies. At concentrations of 5.408 mg/mL and 2.516 mg/mL, cell viability remained at 75.28% and 98.32%, respectively. These results demonstrate that nisin is safe for murine fibroblast cells, maintaining cell viability even at high concentrations. Hence, nisin may represent a promising topical therapeutic alternative for the treatment of canine pyoderma.

KEYWORDS: Cell viability; natural products; MRSP; small animals.

FUNDING: We thank the financial support of Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) – Brazil, grant nº 88887.906349/2023-00.

ULTRASONOGRAPHY AS A TRANSLATIONAL TOOL: THE RELATIONSHIP BETWEEN MORPHOMETRY AND MUSCLE STRENGTH IN SMOKERS

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Introduction: Chronic smoking is linked to reduced muscle mass and contractile tension. However, the precise relationship between muscle morphometry and strength in smokers with preserved pulmonary function remains unclear. As a challenge for translational science, there is a need to identify early and non-invasive biomarkers to assess the systemic impact of smoking. Ultrasonography, a promising tool, can provide valuable insights beyond simple thickness measurements by assessing muscle architecture through the pennation angle (PA), which reflects the organization of muscle fibers. Objective: To investigate the relationship between the thickness and PA measurements of the elbow flexor muscles and handgrip strength in smokers without pulmonary disorders. Methods: This pilot, cross-sectional study, approved by the local Ethics Committee (protocol number: 6.800.983), included 19 current smokers (11 women, 33 [25-46] years old) with preserved pulmonary function (FEV1/FVC and FVC ≥80%). Handgrip strength was measured with a dynamometer, while the thickness and PA of the brachialis and biceps brachii muscles were evaluated using ultrasound (Voluson P8tm, GE Healthcare). Statistical analysis used Pearson's correlation test, with a significance level set at $p<0.05$. Results: Handgrip strength was 75±30% of the predicted value for the patient, while ultrasound measurements showed a relaxed elbow flexor thickness of 3.2±0.6 cm and a relaxed biceps PA of 12.5±2.5º. There was a moderate positive correlation between handgrip strength (%predicted) and relaxed elbow flexor thickness ($r=0.6$; $p<0.01$) and relaxed biceps PA ($r=0.5$; $p<0.05$). No significant correlation was found with measurements taken during muscle contraction. Discussion and Conclusion: These results suggest a significant relationship between peripheral muscle strength and muscle morphology at rest. This finding is clinically relevant as it indicates that ultrasonography can be a non-invasive and translational tool for assessing muscle function in smokers. Measurements of thickness and PA in a relaxed state show promise as effective indicators of muscle strength, potentially aiding in identifying individuals at risk of decline in muscle strength. This study paves the way for the development of early screening and intervention strategies in clinical practice, aimed at minimizing the systemic effects of smoking on musculoskeletal health.

KEYWORDS: strength test; ultrasound; smoking

FUNDING: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

MACROSCOPIC FINDINGS IN CAPTIVE SNAKES FOR VENOM PRODUCTION

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Venomous snakes have great medical and scientific relevance due to the potential of the compounds presents in their venoms for the production of immunobiologicals. The efficiency and high-quality production of venom is directly linked to the health of the snakes, which must be constantly evaluated. The autopsy is an important measure for the sanitary monitoring of snake colonies, allowing the identification of pathological disorders. Therefore, this study aimed to investigate and describe the anatomopathological findings in *Bothrops* and *Crotalus* snakes maintained in captivity in a scientific facility dedicated to venom production between September and December 2024. This study was approved by the CEUA-IBTEC under protocol number013/2024 and the Authorization for Wildlife Management number 0000118253/2024-SP. Thirty snakes, 20 *Crotalus durissus terrificus* and 10 *Bothrops sp.* (eight *B. jararaca*, one *B. pauloensis* and one *B.atrox*), were subjected to autopsy after natural death.The animals were maintained in semi-extensive and intensive breeding systems. Eighteen snakes showing low body condition score and 12 showing normal score, dysecdysis (3/30) and swelling in the caudal third of the body (1/30) were the main findings observed during external inspection. The main macroscopic findings observed at autopsy predominantly involved the digestive system (26/30), including enteritis (8/30), hepatic degeneration (6/30), stomatitis (4/30), esophagitis (4/30) and gastritis (4/30). In the circulatory system, 13 snakes presented pericarditis and one exhibited cardiac hemorrhage. In the respiratory system, pulmonary hemorrhage (3/30), caseous pneumonia (3/30), and verminous pneumonia (1/30), caused by *Rhabdias sp.*, were identified. In the genitourinary system, uric gout (4/30) and ovarian neoplasia (1/30) were observed. These findings may indicate alterations resulting from infections, inflammatory processes and long-time captivity maintenance. It is known that environmental factors, including temperature, humidity, housing conditions, and human presence, may influence the physiological state of snakes, contributing tostress and disease susceptibility.It is concluded that autopsy examination may be a relevant tool for the sanitary monitoring of captive snakes, contributing to the improvement of management practices, animal welfare, and the efficiency of research centers and their production.
KEYWORDS:animal health; autopsy; biopharmaceuticals; venomous snakes.
FUNDING: Secretaria Estadual da Saúde - SES-SP

EARLY MUSCLE DEGREDATION IN SMOKERS: EVIDENCE FROM BICEPS THICKNESS IN PATIENTS WITH AND WITHOUT MILD PULMONARY RESTRICTION

Paffetti, G.S.[1]; Pereira, A.R.[1]; Silva, L.L.[1]; Franco, M.B.[1]; Sanches, P.[2]; Silva, B.[1]; Cabral, R.[1]; Villegas, M.E.[1]; Proença, M.[1]

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Introduction: Smoking causes systemic effects that extend beyond pulmonary function, impacting muscle health. Translational science seeks to identify and mitigate these comorbidities. The loss of muscle mass is a known complication in smokers, but it is still unclear if this muscle degradation manifests early and in parallel with non-obstructive pulmonary dysfunctions. Our study, using ultrasonography, aims to evaluate muscle thickness as a potential biomarker for early systemic degradation in smokers with normal pulmonary function or mild dysfunction. Objective: To evaluate and compare the biceps muscle thickness, at rest and during contraction, in smokers with normal and mild restrictive spirometric patterns. Methods: This cross-sectional study, approved by the Ethics Committee (6.800.983), included 38 smokers (22 women; 39 [25-45] years old; BMI 27 [24-30] kg/m²). Of these, 13 had mild restrictive spirometric patterns. Biceps thickness was evaluated by B-mode ultrasonography with the patient in a supine position. Images were captured at rest and during muscle contraction. Data was analyzed using the Mann-Whitney U test or T-test, with a significance level of $p < 0.05$. Results: Smokers with a mild restrictive pattern had smaller biceps thickness during contraction (1.7 ± 0.3 cm) and a smaller cross-sectional area (5.1 ± 1.2 cm²) compared to smokers with normal pulmonary function (2 ± 0.5 cm and 5.8 ± 1.5 cm², respectively). Statistical analysis revealed a significant difference in contracted biceps thickness between the groups ($p<0.05$), even though pulmonary function was not obstructive. Discussion and Conclusion: Our results suggest that muscle degradation in smokers can occur before severe pulmonary manifestations, even in individuals with normal or only mild restrictive spirometric parameters. This finding is a crucial step in the translational process, as ultrasonography could become a screening tool to early identify the systemic effects of smoking. This paves the way for more effective rehabilitation interventions and follow-ups, focusing on minimizing muscle damage and improving patients' quality of life.

KEYWORDS: Smokers; Ultrasonography**;** Muscle Strength; Respiratory Function Tests

FUNDING: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

HYPOXIC BURDEN IN SMOKERS: A TRANSLATIONAL TOOL FOR ASSESSING CARDIOVASCULAR RISK

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Introduction: Obstructive sleep apnea (OSA) is a well-known determinant of cardiovascular risk (CVR). While the Apnea-Hypopnea Index (AHI) is a standard diagnostic tool, it often fails to capture the full spectrum of nocturnal oxygen desaturations. This poses a challenge for translational science, which aims to develop and validate more sensitive biomarkers for clinical application. The hypoxic burden (HB), a metric quantifying the severity and duration of hypoxemic events, offers a promising alternative. Objective: To verify the hypoxic burden in smokers and its association with cardiovascular risk. Methods: This cross-sectional study included 41 smokers (aged 19-59) with different levels of smoking history. Participants were categorized by spirometry into groups with (PVD) or without (AVD) a ventilatory disorder and by the presence or absence of comorbidities. Anthropometric data and sleep quality (actigraphy and type IV polysomnography) were assessed. Due to the non-normal data distribution, results are presented as median [interquartile range]. The Mann-Whitney and Kruskal-Wallis tests were used for group comparisons, while Spearman's test was applied for correlations. Statistical significance was set at $p<0.05$. Results: The sample had a median age of 40 [30-49] years, a BMI of 27 [24-32] kg/m², and was predominantly female (59%). Participants had a history of mild (32%), moderate (49%), and heavy (20%) smoking, with 61% presenting previous comorbidities. Smokers with ventilatory disorders and comorbidities had a significantly higher HB (36 [14-48]%min/h) compared to those without comorbidities ($p=0.013$) and those with normal lung function and no comorbidities (12 [9-21]%min/h; $p=0.018$). There was a correlation between HB and sleep ODI ($r=0,742$), oxygen saturation time lower than 90% ($r=0,665$) and minimum ($r=0,750$) regardless of the smoking group. Discussion and Conclusion: This study demonstrates that a significant hypoxic burden is already present in smokers with comorbidities, indicating an elevated cardiovascular risk. Our findings validate HB as a clinically relevant translational biomarker that can be easily measured with simplified polysomnography. This highlights its potential as an effective, non-invasive screening tool for early identification and intervention in high-risk patients, addressing a key challenge in clinical practice.

KEYWORDS: Sleepy Apnea Obstructive; Tobacco Smoking; Hypoxia.
FUNDING: CAPES/CNPQ.
ETHICS: 6.800.983.

DEVELOPMENT OF A MULTIFUNCTIONAL PHANTOM FOR QUALITY CONTROL IN MAGNETIC RESONANCE IMAGING (MRI)

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Introduction: Magnetic resonance imaging (MRI) is widely used in diagnostic imaging, allowing detailed tissue evaluations without ionizing radiation. Its use requires rigorous quality control, regulated by ANVISA through RDC No. 330/2019, which make physical tests mandatory to ensure image precision and safety. However, the high cost of commercial phantoms limits their adoption. Objective: To propose a low-cost multifunctional phantom, agile in image acquisition, capable of performing mandatory tests such as SNR, Uniformity, Ghosting, Artifacts, Geometric Accuracy, Slice Thickness and Position Accuracy, and High-Contrast Spatial Resolution. Methodology: The cylindrical object simulates a medium-sized skull (15 cm diameter × 15.5 cm height) filled with a nickel chloride (10 mmol) and sodium chloride (75 mmol) solution, following ACR standards. The differentiated filling optimizes T1/T2 for fields below and above 3 T, with reproducibility and compliance with NEMA, AAPM, and ACR. Inserts are designed for 5 mm slice thickness and 1.5 cm spacing, optimizing protocols in high-demand sectors. A hole in the upper lid allows the insertion of a plastic plug, enabling filling and maintenance, and serves as an artifact test. Crossed ramps (9×9 cm) and calibrated holes ensure slice and geometric accuracy, in addition to guiding the magnet isocenter determination. Two plates with micro-holes (0.9–1.1 mm) filled with water simulate high contrast, fixed with neutral silicone and rubber coating. The lower lid will have eight holes separated by 45°, plus a central one to verify known center-to-center dimensions, ensuring reproduction of real sizes in 2D MRI. Upper and lower lids incorporate multiple tests, compacting and reducing costs. SNR, Uniformity, and Ghosting are measured in homogeneous areas of the solution. Results: The phantom reduces costs by 98.5% compared to commercial models (R$ 54,105.70), with acquisition time around 15 minutes. It complies with national regulations (IN No. 97/2021), democratizing access to quality control, promoting diagnostic excellence in diverse clinical services. Discussion: The device integrates key MRI quality tests, translating technical knowledge into practical application. Conclusion: The proposal proves to be concise, offering a viable solution to democratize access to quality control in MRI, thereby contributing to the safety and reliability of diagnostic imaging in Brazil.
KEYWORDS: Quality control; low-cost phantom; medical diagnostics.
FUNDING: This study did not receive any funding.

EXPRESSION OF SARS-COV-2 CO-RECEPTORS IN MURINE MELANOMA: IDENTIFICATION OF POTENTIAL NEW MELANOMA TARGETS

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Melanoma is an aggressive type of skin cancer with high metastatic potential. Current therapeutic approaches are often insufficient to achieve durable responses in advances melanoma cases, and, therefore the identification of new targets is desired for the study of melanoma. Cells within the tumor environment have a close entangled relationship, generating the production of many factors and molecules that support the development and progression of melanoma. The aim of this study was evaluating the expression of the SARS-CoV-2 co-receptors in murine melanoma. An active search was conducted using public mRNA seq database available for B16F10 murine melanoma. Furthermore, one of the genes analyzed was chosen and used in qPCR approach to validate differential expression between normal skin and melanomas from C57BL6 mice. The ITGB1, ITGB3, HSPA5, BSG/CD147, VIM genes were widely expressed with expression in cancer associated-fibroblasts (CAFS), melanoma cells, dendritic cells, macrophages, tumor T cells, NK cells and B cells. Otherwise, the genes DPP4, AXL, TLR4, HSPG2 and NRP1 displayed a more restricted expression with predominance in fibroblasts, endothelial cells and CAFs within melanoma. Gene expression analysis demonstrated that the NRP1 gene was increased in murine melanomas in comparison with normal skin, with a significant increase in melanoma derived from D4M-3A cells. Our preliminary data indicate that some SARS-CoV-2 co-receptor genes may display increased expression in the melanoma microenvironment, representing potential targets to be explored in future studies of this type of cancer.

KEYWORDS: B16F10, SARS-CoV-2 coreceptors; melanome, neuropilin

FUNDING: This work was supported by CAPES -Institutional Internalization Program - PrInt (Grant 88887.840186/2023-00); the Center for Translational Science and Biopharmaceutical Development (FAPESP Grant 2021/11936-3) and the Minas Gerais Research Foundation – FAPEMIG, the Minas Gerais Network for Translational Research in Immunobiologicals and Biopharmaceuticals in Cancer (REMITRIBIC, RED-00031-21).

EVALUATION OF THE EFFICACY OF CANNABIS OIL COGNITIVE DYSFUNCION SYNDROME THROUGH THE (CADES) SCALE: PRELIMINARY RESULTS

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Canine cognitive dysfunction syndrome (CCDS) is a neurodegenerative condition analogous to Alzheimer's disease (AD) in humans, characterized by anxiety, disorientation, alterations in sleep, behavior, and social interaction, affecting approximately 14% to 35% of elderly dogs. The dog represents a relevant translational model for the study of human diseases, as they share the same environment and habits, reinforcing its experimental value in the study of both CCDS and AD. In this context, the present study aims to demonstrate the improvement of cognitive clinical signs, through the application of the canine dementia scale (CADES), before and after administration of full-spectrum Cannabis oil in the treatment of elderly dogs diagnosed with CCDS, attended at the Veterinary Neurology Service of FMVZ-Unesp-Botucatu. Diagnosis was carried out through the application of the canine dementia scale (CADES), allowing the classification of patients into different degrees of cognitive impairment (mild, moderate, and severe), as well as clinical and neurological evaluation, magnetic resonance imaging, cerebrospinal fluid collection, and the application of the episodic-like memory test (TEMEC). So far, nine dogs treated with Cannabis oil have shown significant improvement in cognitive impairment scores, while none of the seven dogs in the placebo group demonstrated positive progression. Preliminary results suggest that full-spectrum Cannabis oil may act on the modulation of the endocannabinoid system, reducing clinical signs of CCDS and promoting improvement in the quality of life of patients and their tutors.. This difference suggests that the compound may act on the modulation of the endocannabinoid system, contributing to the reduction of clinical signs of cognitive dysfunction. Moreover, the contrast between groups reinforces the therapeutic potential of Cannabis oil as a promising alternative for managing cognitive decline in elderly dogs, with a direct impact on the quality of life of patients and their caregivers. However, the completion of the study is necessary to confirm the initial findings.

Keywords: Alzheimer's; dementia; translational model.
Protocol CEUA 000.113/2024.

AREA: Health Sciences
SUBAREA: Public Health

GOVERNANCE AND COMPLIANCE POLICY IN CENTERS FOR DRUG DEVELOPMENT AND PRODUCTION

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Introduction: Clinical research and the development of biopharmaceuticals are strategic pillars of public health policy. In this context, structures that integrate education, research, and production are essential to Translational Science. The V-BioPharma School-Factory, from CEVAP-UNESP, is an innovative initiative that produces biopharmaceuticals according to Good Manufacturing Practices (GMP). In a highly regulated sector, Corporate Governance and Compliance practices are crucial to ensure institutional integrity, transparency, and sustainability. Objective: To develop a Governance and Compliance Policy customized for the CEVAP/UNESP Biopharmaceutical Factory, based on ethical, legal, and institutional principles. Methodology: Descriptive study with a quantitative approach, based on document analysis and public data. A questionnaire was developed and applied to five public and five private institutions active in biopharmaceutical research and development. Results: Private institutions showed stronger practices in financial transparency, internal communication, and human capital development. Both sectors demonstrated commitment to mission, vision, and accountability. National and international regulations and regulatory agencies were identified. Discussion: The effectiveness of practices is more closely related to organizational culture than to the mere existence of formal norms. The private sector operates with greater normative clarity, while the public sector faces challenges such as bureaucracy and limited access to information. The proposed model is adaptable, considers the context of public administration, and integrates training, communication, and institutional engagement actions. Conclusion: The developed policy strengthens integrity and efficiency, promoting an institutional culture based on ethics and social responsibility. This model may serve as inspiration for other public initiatives in science, health, and innovation. Outputs: Ten regulatory instruments were developed, including codes of conduct, internal regulations, engagement policies, and a whistleblower channel.
KEYWORDS: Corporate Governance; Compliance; Public-private partnership
FUNDING: Coordination for the Improvement of Higher Education Personnel (CAPES)

RECTUS FEMORIS CROSS-SECTIONAL AREA IN SMOKERS: A SEARCH FOR EARLY BIOMARKERS OF MUSCLE DYSFUNCTION USING ULTRASONOGRAPHY

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Introduction: Smoking causes systemic effects, significantly impacting the musculoskeletal system. Translational science calls for the identification of accessible tools to diagnose and treat these dysfunctions before they progress. The rectus femoris cross-sectional area (RFCA) is a recognized predictor of muscle function. Objective: To compare the RFCA in smokers with and without ventilatory disorders and to investigate the relationship between ventilatory status and ASTrf reduction. Methods: This cross-sectional study evaluated 87 adult smokers. We assessed smoking history and respiratory function via spirometry. RFCA measurements were performed using B-mode ultrasound. Participants were divided into two groups: with and without ventilatory disorders. Statistical analysis compared the ASTrf between groups and assessed its correlation with smoking history. The project was approved by the local Ethics Committee (number: 6.800.983). Results: No significant difference in ASTrf was found between smokers with and without ventilatory disorders ($p>0.05$). Similarly, there was no significant correlation between ASTrf and smoking history ($p>0.05$). Discussion and Conclusion: Our findings suggest that, in smokers with mild-to-moderate pulmonary dysfunction, the RFCA alone may not be a sufficiently sensitive biomarker to detect early muscle damage. This highlights a crucial challenge in translational research: to identify other, more subtle ultrasound markers. We propose that future studies should investigate factors such as intramuscular fat accumulation and fibrosis, which may precede significant muscle mass loss. The study reinforces the potential of ultrasonography as a valuable tool for physiotherapeutic clinical practice, offering an economical and rapid method to guide more effective, effective, early interventions and aprroaches to managing the systemic health effects of smoking.

KEYWORDS: spirometry; ultrasound; smoking
FUNDING: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

INTEGRATING TECHNOLOGY IN HEALTH: OBJECTIVE PHYSICAL ACTIVITY ASSESSMENT IN SMOKERS AND ITS TRANSLATIONAL POTENTIAL

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Introduction: Smoking is a known risk factor for numerous chronic diseases and also linked to reduced functional capacity. While spirometry is the standard for assessing lung function, it doesn't provide a complete picture of an individual's daily physical activity profile or time spent in sedentary behavior, both crucial for systemic health. This gap represents a key challenge for translational science, which aims to integrate objective tools like accelerometers to identify lifestyle behaviors that compromise smokers' well-being, even before the onset of established lung dysfunction. Objective: Evaluate the level of physical activity (PA) and sedentary behavior (SB) of adult smokers participating in a cessation program and to compare the results with World Health Organization (WHO) recommendations to guide health interventions. Methods: This cross-sectional study evaluated 40 smokers (22 women; 38±11 years old) with no lung dysfunction confirmed by spirometry. PA and SB were monitored for 7 days using three-axis accelerometers (ActiGraph wGT3X-BT) and pedometers (YAMAX PW-611). Daily steps, time in sedentary behavior (min/day), and time in moderate-to-vigorous physical activity (MVPA in min/day) were quantified. The project was approved by local Ethics Committee (CAAE: 14769419.3.0000.5402). Results: The participants showed a predominantly inactive profile, spending an average of 78±5% of their waking time in sedentary behavior (864±221 min/day). The average number of steps per day was 7,523±2,701 (accelerometer). The average time in MVPA was only 28±14 min/day. Consequently, the majority of participants (72% according to accelerometry) did not meet the WHO recommendation of 150 minutes of MVPA per week. Discussion and Conclusion: Our findings, obtained through an objective and non-invasive assessment, demonstrate that adult smokers have a predominantly sedentary lifestyle and do not meet physical activity recommendations, even in the absence of apparent spirometric changes. These results have significant translational potential. They highlight the need for a comprehensive approach in smoking cessation programs that goes beyond treating nicotine dependence and integrates the promotion of a more active lifestyle. By using technologies like accelerometers to bridge the gap between clinical assessment and real-world behavior, this study contributes to the development of more effective and holistic health interventions, ultimately improving systemic health.

KEYWORDS: smoking; sedentary behavior; accelerometry
FUNDING: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

EARLY CHANGES IN DIAPHRAGMATIC THICKNESS IN SMOKERS: A TRANSLATIONAL APPROACH FOR RESPIRATORY PREVENTION

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Introduction: Smoking is a significant risk factor for chronic obstructive pulmonary disease (COPD), a condition associated with substantial changes in the respiratory muscles, especially the diaphragm. Translational science emphasizes the early identification of subclinical abnormalities to guide preventive interventions. Studies have shown modifications in diaphragmatic architecture in smokers, even without respiratory dysfunction. Our study aims to validate ultrasonography as a translational tool for identifying these early morphological changes before the onset of chronic lung disease. Aim: To compare the diaphragmatic thickness between smokers without respiratory dysfunction and matched non-smoking controls. Methods: This cross-sectional study was approved by the local Research Ethics Committee (protocol number: 6.800.983). We included 65 active smokers (39 women; 39[31-49] years; BMI 28±6 kg/m^2; 15[6-29] pack years), and 25 healthy non-smokers (15 women; 40±10,5 years; BMI 25[23-30]kg/m^2). Diaphragmatic thickness was evaluated with a high-frequency linear ultrasound transducer in B-mode, at the eighth and ninth intercostal spaces. Measurements were taken at total lung capacity (TLC) during inspiration and residual volume (RV) during expiration. Spirometry was used to identify respiratory dysfunction. The Mann-Whitney U test was used for group comparisons, with significance at $p<0.05$. Results: Considering inspiratory thickness measurements, the smoking group had a median of 0.25 [0.21-0.31] cm, while controls had 0.23 [0.14-0.28] cm. For expiratory thickness, smokers had a median of 0.16 [0.13-0.19] cm and controls had 0.14 [0.11-0.17] cm. Significant differences were indicated between the groups for inspiratory ($p = 0.04$) and expiratory ($p = 0.03$) thickness. Discussion and Conclusion: These findings suggest that, even without apparent respiratory dysfunction, smoking can impact diaphragmatic morphometry, as expressed by its thickness. These findings reinforce the use of ultrasonography as an effective translational tool for clinical practice. It allows for the early and non-invasive detection of muscle changes in at-risk populations, paving the way for targeted preventive strategies before the progression to symptomatic respiratory diseases like COPD.

KEYWORDS: Smoking; Ultrasonography; Diaphragm.
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SECRETION OF AMAZONIAN FOREST FROGS AS A SOURCE OF ANTIMICROBIAL SUBSTANCES

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Bacterial resistance has consolidated itself as one of the greatest threats to global health, leading to increased morbidity, mortality, and treatment costs. It is estimated that by 2050, infections caused by multidrug-resistant bacteria could cause more deaths than cancer. Parallel to the advancement of this resistance, there is a slow and unsatisfactory pace in the discovery and development of new antimicrobials, making the identification of innovative and effective molecules urgent. Bioprospecting emerges as a promising strategy in this scenario, by exploring bioactive compounds present in nature with therapeutic potential. Among the most relevant sources are anurans, whose skin secretions are rich in peptides and proteins with diverse biological activities. This study aims to identify components with antimicrobial potential in the venoms of the species *Phyllomedusa bicolor, Phyllomedusa camba*, and *Pithecopus palliatus*. Antimicrobial activity was evaluated against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* by agar disk diffusion and determination of the minimum inhibitory concentration (MIC). The results indicated that the venoms of *P. bicolor* and *P. camba* showed significant antimicrobial activity. The venom of *P. bicolor* exhibited antimicrobial action against *E. coli* (MIC of 0.625 mg/mL), *S. aureus* (2.5 mg/mL), and *P. aeruginosa* (1.25 mg/mL). *P. camba* inhibited *E. coli* (0.625 mg/mL) and *S. aureus* (1.25 mg/mL), with a moderate effect on *P. aeruginosa*. *P. palliatus* showed no activity at the tested concentrations (up to 1 mg/mL). The findings demonstrate the potential of anuran venoms from the family *Phyllomedusidae* as sources of new molecules with antimicrobial activity. Although still in the initial phase, the results justify the continuation of the project with fractionation and purification steps of the active secretions, aiming to identify the compounds responsible for the antimicrobial action. This study highlights the relevance of Amazonian biodiversity in the search for new drugs.

KEYWORDS: *Phyllomedusa bicolor*, kambo, *Pithecopus palliatus*, *Phyllomedusa camba*, antimicrobial

INHIBITION OF BIOFILM GENE EXPRESSION IN *PSEUDOMONAS AERUGINOSA* BY ZNO NANOPARTICLES: A TRANSLATIONAL APPROACH

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Introduction: *Pseudomonas aeruginosa*, a Gram-negative bacterium with multiple virulence factors and high antimicrobial resistance. Biofilm formation is the principal resistance mechanism observed. Therefore, there is growing interest in seeking alternative therapies for the treatment of highly resistant bacterial infections. In this context, zinc oxide (ZnO) nanoparticles exhibit stability, low environmental impact, and potential to inhibit biofilm formation and disrupt already formed biofilms. Objectives: This study aimed to standardize RT-qPCR for the assessment of expression of the algC and pslA genes, which are involved in the formation, maturation, and maintenance of P. aeruginosa biofilms, and to investigate the effect of nanoparticles on this resistance mechanism. Methodology: Strain of *Pseudomonas aeruginosa* ATCC 27853 was cultured for 24 hours in Mueller-Hinton agar. The inoculum was standardized according to the McFarland scale, 0.5 (10^8 UFC/mL). To the inoculated flasks, ZnO nanoparticles at a MIC50 concentration were added, and the flasks were placed on a shaker for 24 hours at 37°C. After this period, the flasks were centrifuged and the cells subjected to total RNA extraction using Trizol. The extracted material was quantified and allocated for amplification by quantitative PCR. The genes analyzed were *algC*, *pslA* and 16S as an endogen gene. Results: Sample-treated and untreated samples were compared using the relative expression method ($2^{-\Delta\Delta Ct}$). Nanoparticle-treated samples showed mean values of approximately 0,425 ± 0,066 (*algC*) and 0,208 ± 0,366 (*pslA*), while untreated samples showed means of 9,318 ± 1,716 (*algC*) and 5,502 ± 3,096 (*pslA*). Discussion: The exposure of biofilms to ZnO nanoparticles showed a significant reduction in the expression of algC and pslA. The confirmation of effectiveness was achieved by comparing treated and untreated samples, whose difference was statistically significant by ANOVA analysis (algC: p = 0,0008; pslA: p = 0,0181). Conclusions: ZnO nanoparticles show potential to inhibit *P. aeruginosa* biofilm formation by interfering with the expression of key genes involved in extracellular polysaccharide production. The findings support the translational development of alternative therapies for *P. aeruginosa* infections, contributing to strategies that target biofilms as a therapeutic target.

KEYWORDS: *Pseudomonas, aeruginosa, biofilm, RT-qPCR*
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FREEZE-DRIED VENOM OF *CROTALUS DURISSUS TERRIFICUS*: COMPARISON OF THE CHROMATOGRAPHIC PROFILE OF FIVE YEARS

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Introduction: Freeze-drying is a dehydration technique in which the product is frozen, under vacuum, and the water is sublimated. This increases the stability and shelf life of proteins and biomolecules of pharmacological interest. Freeze-dried snake venoms have enabled long-term storage of these proteins without significant concerns regarding availability or stock shortages, relevant for large-scale biomedical research and biopharmaceutical production. Such applications include the manufacture of Heterologous Fibrin Biopolymer (HFB), created by CEVAP and planned for production and distribution by CDMO V-Biopharma for future phases of Clinical Research, in accordance with Good Clinical Practices (GCP) and ANVISA regulations. *Crotalus durissus terrificus* (Cdt) venom contains the main toxins crotoxin, gyroxin, convulxin, and crotamine, with gyroxin being the ideal molecule for HFB. Objective: The aim was to compare the chromatographic profiles of lyophilized Cdt venoms from different storage periods - older (2016, 2017, 2018) and recent (2024, 2025) samples - obtained from CEVAP, to observe whether significant changes in the peaks of the main toxins and proteins occur during these years. Methodology: Chromatographic analysis was performed using a Shimadzu® LC-20AT reversed-phase HPLC system, C18 column. 10 mg of lyophilized venom was solubilized in 1 mL of trifluoroacetic acid (TFA). Elution was performed with solvent A (0.1% TFA) and B (0.1% TFA, 90% acetonitrile) at a flow rate of 1 mL/min, with absorbance at 214 nm. Results: The comparison shows that crotamine peaks in the 2017 and 2018 samples were significantly lower than in the other years. This variation is about biochemical differences in the sample: crotamine is a peptide that could be present in Cdt or not, and the venom analyzed was Introduction: Frea pooled sample, the proportion of crotamine influenced the quantitative difference. Crotoxin peaks (crotapotin and phospholipase A_2) showed minimal variation in all years, while the 2016 sample had a comparatively higher gyroxin peak. Discussion: Overall, freeze-dried Cdt venoms, both older and more recent, showed no substantial changes in protein composition, other than variability in crotamine. Conclusion: These findings indicate that storage of freeze-dried venom for up to nine years does not result in significant protein degradation, supporting the reliability of long-term venom preservation for biomedical research and pharmaceutical applications.

KEYWORDS: Freeze-dried venom; Snake venom; Chromatographic profile of snake venom; Crotalus durissus terrificus.

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PROTEOMIC ANALYSIS OF PLASMA FROM PATIENTS WITH *CROTALUS* SNAKEBITE ENVENOMING REVEALS POTENTIAL BIOMARKERS AND MECHANISMS ASSOCIATED WITH CLINICAL SEVERITY

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Abstract: Snakebite envenoming is a serious public health issue. In Brazil *Crotalus* venom causing systemic changes that can lead to severe complications. The severity of envenoming is classically determined by the manifestation of signs and symptoms. However, the identification of molecular biomarkers is crucial for predicting clinical evolution and aiding in therapeutic management. This study aimed to characterize proteomic alterations in the plasma of patients envenomated by *Crotalus* according to clinical severity. Plasma proteomics was used to compare the protein profiles of patients with moderate (C-Mod) and severe (C-Sev) *Crotalus* snakebite to a healthy control group (HC). Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) were applied to explore the separation between the groups, and based on VIP scores, we identified relevant proteins for discrimination. The relative abundance of proteins was compared between groups and classified into clusters according to their abundance patterns in response to the severity. Finally, a functional enrichment analysis was performed via Metascape to identify the affected biological pathways. PCA and PLS-DA analyses revealed a clear separation between envenomated patients and the control group, confirming that *Crotalus* snakebite causes significant alterations in the plasma proteome. The most relevant proteins for this discrimination, based on the VIP score, include C8G, SERPINF2, PLG, VTN, FGB, among others. The distinction between the C-Mod and C-Sev groups was more subtle, yet statistically significant, indicating that the progression of severity is associated with an exacerbation of protein expression patterns. We identified 35 proteins that showed a progressive increase in abundance with severity, such as APOD, CPB2, CP, APOE, and F9. On the other hand, 28 proteins, including FGG, CFB, VTN, PLG, and HRG, exhibited a progressive reduction in abundance. The functional enrichment analysis of these proteins revealed that the most impacted pathways are the complement and coagulation cascades, platelet degranulation, and inflammatory response. The high relevance of these pathways confirms that the dysregulation of the coagulation and complement systems is the primary mechanism for the progression of severity. The identification of biomarkers can assist in risk stratification and the development of more targeted diagnostic, prognostic, and therapeutic strategies.

KEYWORDS: Biomarkers of severity; *Crotalus* venom; Plasma proteomics; Snakebite envenoming.

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