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Biomedical Research Poster Abstracts: *Featuring Students and Faculty from the University of Hawaii at Hilo*

Monday, January 6th 2020 - 6:30 pm to 8:00 pm

6th International Workshop on Angiostrongylus and
Angiostrongyliasis

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Preliminary Guidelines for the Diagnosis and Treatment of Human Neuroangiostrongyliasis (Rat Lungworm Disease) in Hawaii

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In 2016 in response to an apparent increase in the number and severity of cases of neuroangiostrongyliasis in Hawaii, Governor David Ige appointed a Joint Task Force on Rat Lungworm Disease. A Clinical Subcommittee of the Task Force that included tropical medicine and infectious disease specialists and experts in parasitology and immunology was formed to develop clinical practice guidelines to address the diagnosis and treatment of the disease. The group reviewed in detail the relevant literature in humans and animals and consulted with national and international experts to formulate Preliminary Guidelines for the Diagnosis and Treatment of Human Neuroangiostrongyliasis (Rat Lungworm Disease) in Hawaii.

The guidelines were published in August 2018. We summarize the Key Points in the document and emphasize that treatment of neuroangiostrongyliasis with ***albendazole*** appears to be:

- 1) *SAFE when combined with high dose corticosteroids and*
- 2) *EFFECTIVE if given within the first 7-14 days after infection.*

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Natural Product Drug Discovery for An Emerging Parasitic Nematode Disease

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Hawaii leads the nation in reported cases of neuroangiostrongyliasis, or rat lungworm disease, a potentially lethal central nervous system infection caused by the parasitic nematode *Angiostrongylus cantonensis*. Humans are infected following ingestion of L3 (infectious third-stage) larvae-harboring snails or slugs. Antihelminthic drugs that selectively inhibit the migration of parasite larvae to the brain are unavailable. We have established a semi-pure natural products library from a collection of unique Hawaiian microorganisms, developed a system for rearing semi-slugs to provide infectious L3 larvae, and designed a novel approach to conduct high-throughput screening of natural products for motility-inhibition bioactivity against *A. cantonensis* larvae. The efficacy of natural products will be tested on infectious *A. cantonensis* larvae. Motility-inhibition bioactive natural products will be identified and characterized through assay-guided separation and structure elucidation.

Morphometric measurements of third-stage juveniles of the rat lungworm (*Angiostrongylus cantonensis*) in Hilo, Hawaii.

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Rat lungworm, Angiostrongylus cantonensis, is a challenging human-parasitic nematode of the tropics. Morphological identification of the third-stage juvenile (J3) of *A. cantonensis* is an additional challenge because of scarce and difficult to access publications on their morphometric parameters. Therefore, a morphometric study of J3 of *A. cantonensis* was conducted at the University of Hawai'i at Hilo. The nematodes were heat-relaxed on slides and morphometric parameters were measured immediately by means of a calibrated ocular micrometer under an inverted microscope (n=7). Mean \pm standard error of the mean for each parameter was calculated. Nematode body length, maximum width, length of esophagus, anus to tail length and excretory pore from anterior were $473.57 \pm 15.26 \mu\text{m}$, $26.04 \pm 0.76 \mu\text{m}$, $171.67 \pm 3.76 \mu\text{m}$, $35.50 \pm 1.82 \mu\text{m}$ and $91.88 \pm 4.04 \mu\text{m}$, respectively. The length and width of the rod-like structure at the mouth (rhabdion) were $18.3 \pm 2.13 \mu\text{m}$ and $4.06 \pm 0.4 \mu\text{m}$, respectively. Additional parameters: nematode body length/greatest body diameter (a), body length/distance from anterior to esophago-intestinal valve (b) and body length/tail length (c) were 18.39 ± 0.71 , 2.78 ± 0.07 and 13.26 ± 0.73 , respectively. The shape of the nematode tail was conical. These morphometric parameters could be used in identification of J3 of *A. cantonensis*.

Angiostrongyliasis in 2020: areas of clinical research need and interest

Chad Meyer, MD, MSc, DTMH

1. Spectrum of Disease severity
2. Prevalence of mild disease
3. Anti parasitic interventions including immediate albendazole after known / accidental exposures
4. Urgent need for rapid diagnostics
5. Chronic disease presentation and treatment issues
6. Prevention strategies (freezing apple snails in SE asia and China vendor markets)
7. Review of confirmed Ac cases in Hawaii; acute and long term outcomes

The presentation process will permit (and promote) real time additions from attendees viewing the poster. It is hoped the format will illustrate the breath of research needs, and will promote research collaboration amongst the various fields of interest.

How to differentiate between *Angiostrongylus cantonensis* and *Gnathostoma spinigerum* in eosinophilic meningitis?

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Background: *Angiostrongylus cantonensis* and *Gnathostoma spinigerum* are the two most common causative agents for eosinophilic meningitis. Treatment is totally different for both parasites. Serological test is the most useful test to differentiate between these two parasites. Clinical factors may be helpful but limited. This study aimed to evaluate if clinical factors can be used to differentiate causes of eosinophilic meningitis between the two parasites.

Methods: We reviewed reported patients diagnosed as eosinophilic meningitis from either *A. cantonensis* or *G. spinigerum* from literatures. The diagnosis must be confirmed either by serological tests or pathologically. Clinical factors between the two groups were analyzed by descriptive statistics and multivariate logistic regression analysis.

Results: There are 155 definite cases of eosinophilic meningitis caused by these two parasites; 131 cases (84.5%) were in *A. cantonensis* group. There were 11 significant different factors between both groups by descriptive statistics such as incubation period, paresthesia, or xanthochromic spinal fluid. Only two factors were independently associated with *A. cantonensis* group; radicular pain and motor weakness. The adjusted odds ratio (95% CI) of both factors were 0.015 (0.001, 0.204) and 0.020 (0.003, 0.147).

Conclusion: Presence of radicular pain and motor weakness were suggestive of Gnathostomiasis in eosinophilic meningitis patients.

Who will have longer duration of headache in serologically-proven eosinophilic meningitis patients if left untreated?

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Abstract

Acute severe headache is the main presentation of eosinophilic meningitis caused by *Angiostrongylus cantonensis*. Oral corticosteroid is effective in reduction of duration of headache but may be contraindicated in particular patients. This study investigated clinical factors associated with duration of eosinophilic meningitis caused by *A. cantonensis* if left untreated. We conducted a retrospective study at a University Hospital, Thailand. The study period was between 1997-2018. The inclusion criteria were adult patients who diagnosed as eosinophilic meningitis, had positive serological test for *A. cantonensis*, received only supportive treatment, and had documented of complete clinical course. Factors associated with duration of headache were executed by multivariate linear regression analysis. There were 55 eosinophilic meningitis patients who received placebo treatment; one patient was excluded due to incomplete clinical data. In total, there were 54 patients in the final analysis. Of those, 39 patients (79.22%) were male and the mean (S.D.) age of all patients was 33.72 (12.22). All patients presented with acute severe headache. The mean (S.D.) duration of headache after treatment with placebo was 16.04 (12.37) days and range of 1-49 days. The only factor associated with duration of headache was sex. The male sex had a coefficient of -8.37 (95% CI: $-16.17, -0.56$; p value 0.036). The medians duration of headache in male and female patients were 11 and 20 days, respectively. In conclusion, sex was a significant factor associated with duration of headache in eosinophilic meningitis from *A. cantonensis*.

Key words: eosinophilic meningitis; *Angiostrongylus cantonensis*; predictors; headache

A national epidemiological data of eosinophilic meningitis of Thailand

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Background: Thailand is the most prevalent country of eosinophilic meningitis. However, there is lack of epidemiological data of eosinophilic meningitis. This study aimed to review and report epidemiological data of eosinophilic meningitis in Thailand.

Methods: Epidemiological data of Thailand were retrieved from the Center of Epidemiological Information, Bureau of Epidemiology, Ministry of Public Health, Thailand. The study period was between 2014 and 2019. Data were presented by descriptive statistics.

Results: There were 1,092 cases of eosinophilic meningitis during the study period. The average number of patient per year was 182 cases or 0.27 cases/100,000 population. Male patient accounted for 66%. The most common age groups were 35-44 years (21.25%) and 45-54 years (20.97%). There were 2 cases with age under 1 year. The months with prevalent number of eosinophilic meningitis were between September and January; over 100 cases. The common areas of eosinophilic meningitis were upper part of Northeast, Thailand; 65.38%. The three most common occupations were farmer (44.68%), laborer (27.38%), and students (13.73%), respectively. The mortality rate was zero.

Conclusions: Farmers and laborers are occupation at risk for eosinophilic meningitis. It affects working-aged persons but can also infected in young patients. The mortality rate was zero.

Development of a Sero-Diagnosis Technique for *Angiostrongylus cantonensis* Using Enzyme-Linked Immunosorbent Assay

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Angiostrongylus cantonensis is a pathogenic, zoonotic nematode that can cause human eosinophilic meningitis, known as neuroangiostrongyliasis or rat lungworm disease. It is considered an emerging global infectious disease and its epicenter in the US is East Hawaii Island. Clinical presentation ranges from mild flu-like symptoms (headache, vomiting, nausea, and paresthesia) to severe (paralysis, blindness, and/or fatal meningitis).

Definitive diagnosis is currently achieved through detection of whole *A. cantonensis* larvae in cerebrospinal fluid (CSF), or PCR to detect parasite DNA in CSF. This research aims to develop an indirect Enzyme Linked Immunosorbent Assay (ELISA) of human serum to detect antibodies produced as a response to 31kDa antigen isolated from Hawaii *A. cantonensis*. The clinical benefit of developing a blood test would be an expedited, dependable, affordable, and minimally invasive alternative to current standards.

Statistical analysis is not complete, but preliminary data suggests the Hawaii 31kDa ELISA could become a viable diagnostic tool. Of a pool of 151 volunteers that donated blood samples, 12 individuals identified themselves as diagnosed by a clinician. Volunteers recently diagnosed (>2014, n=7) had a mean ELISA value (EV) of 68.57%. Those diagnosed with older infections (<2014, n=5) had a mean EV of 34.77%. The mean EV of participants who reported no clinical diagnosis of *A. cantonensis* (n=139) was 25.42%. Overall, the Hawaii (HI) 31kDa proteins showed a seroprevalence in the volunteers of 25% (n=151).

Further testing, including cross-reactivity studies, may allow us to develop an effective, affordable, and quick diagnostic tool for clinicians and diagnostic laboratories.

Co-authors: Kirsten Snook, Yaeko Tagami, Kathleen Howe, Steven Jacquier, Kirsten Cannoles, Stefano Quarta, Lisa Kaluna, Praphathip Eamsobhana, Susan Jarvi.

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Evaluation of Antioxidant and Anti-Cancer Activity of Fucose-Containing Sulfated Polysaccharide from Marine Algae

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Fucoidan, a sulfated polysaccharide purified from brown algae, possesses a variety of pharmacological effects, including anti-inflammatory, antioxidant, and anticancer properties; however, different extraction method affect the biological activity and type of extracted fucoidan. The objectives of this study were to extract crude fucoidan and evaluate the medicinal properties found in *Undaria pinnatifida* and dried kelp with the aim to ascertain whether those have these activities, as well as which functional groups confer them. *U. pinnatifida* and dried kelp are common brown algae species eaten in soups. Both are easily accessible to consumers and are believed to have health benefits. Both specimens were pre-treated and extracted using two methods - the conventional method and a microwave-assisted extraction. Following extraction, samples were evaluated for antioxidant activity using the ferric ion-reducing antioxidant power (FRAP) assay. The structure of fucoidan was analyzed using nuclear magnetic resonance (NMR), and Fourier Transform Infrared Spectroscopy. Extracted fucose was tested for cytotoxicity using an *in vitro* sulforhodamine B (SRB) assay against human breast (MCF-7) and lung (Lu-1) cancer cell lines. Analysis of data revealed activities with inhibitory concentrations greater than 50% against several cancer cell lines. These results demonstrated fucoidan isolated from *U. pinnatifida* and dried Kelp does possess antioxidant and anti-cancer properties.

Anti-inflammatory and antimicrobial properties of *Moringa oleifera* fruits

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Moringa oleifera (Mo) Lam. (Moringaceae), commonly known as ‘Malunggay’ in Hawaii and also known as the drumstick tree, grows quickly and is resistant to droughts; it is native to South-east Asia. The Mo possesses health benefits such as antioxidant and anti-inflammatory activities, and probable cholesterol lowering properties. Different parts of this plant contain a profile of phytochemicals and are a good source of amino acids, beta-carotene, protein, vitamins, and various phenolic compounds. The seeds and different parts of the plant have been used in folk medicine to treat various conditions and inflammation.

Objective: Our aim is to prepare Mo fruit extract and evaluate its the antimicrobial and anti-inflammatory activities.

Methods: (a) Extraction: *Moringa oleifera* (Mo) fruits were separated from their shells. The shells and fruit were separately grounded in glass mortar and pestle to form a powder. Fruits yielded 290 g and shells yielded 116 g of powder. Extraction was done using methanol thrice. Solvents were then evaporated to yield fruit extract 75 g and shell extract 16 g. These extracts and one previously isolated compound (4-[4'-*O*-acetyl- α -L-rhamnosyloxy]isothiocyanate) from Mo fruits were subjected for bioassays. (b) Antimicrobial assay: Modified Kirby-Bauer method was used for testing against *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *S. aureus* (USA300 LAC). In brief, overnight culture of bacterial strain was inoculated on a Mueller-Hinton (MH) agar plate. Next, samples with known concentrations were added onto 6-mm filter paper disk on the MH agar plate. The plates were incubated at 35°C for overnight. Samples with clear zone of inhibition (ZOI) more than 8 mm were considered active with antimicrobial activity. (c) Anti-inflammatory assay: Extracts will be tested for the potential to inhibit TNF- α -induced NF- κ B activity using stably transfected human embryonic kidney cells NF- κ B Luc-293.

Results: The preliminary screening showed that the Mo fruit seed extracts were active against *S. aureus*. The minimum inhibitory concentration (MIC) value determination for *S. aureus* is currently in progress.

Summary: Our preliminary study support that the Mo fruit contains bioactive compounds with antimicrobial activity. These extracts and a compound, namely, 4-[4'-*O*-acetyl- α -L-rhamnosyloxy]isothiocyanate showed inhibition activity against *S. aureus* strain. Anti-inflammatory activity of these samples are currently in progress. Thus, these plants seem worthy of continued study.

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Oxidation of Phosphonic Acids

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ABSTRACT

Phosphonic acids are in widespread use as herbicides in Hawai'i and around the world, the best known example being glyphosate. It is well appreciated that glyphosate degrades in the environment to form ortho-phosphate by oxidation of a Carbon-Phosphorus bond. Our laboratory has modelled the mechanism of this process by studying the oxidation of methyl phosphonic acid (MPA), which has a simpler structure than glyphosate. MPA was stirred with oxides of metals commonly found in soils: iron, manganese, copper, zinc and aluminum in water and in the presence of acid and base. After stirring for several hours, the solutions were filtered and analyzed for ortho-phosphate using the molybdenum blue assay. The most active metal oxide was manganese dioxide. Differences were observed between Copper (II) oxide and copper (III) oxide and between acidic and basic solutions. MPA, phenyl phosphonic acid and benzyl phosphonic acid in water, and in the presence of one equivalent of base, were exposed to UVC light (254 nm, 4 hours, Ray-O-Net lamps), in aerated water, and the percent conversion to ortho-phosphate was determined by the molybdenum blue method. Photochemical oxidation in the presence of 3% hydrogen peroxide was also investigated, in the presence and absence of base.

Keywords: phosphonic acids,

Themes: mechanisms of oxidation

In Vitro Expression and Analysis of Centralspindlin

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Rationale: Cell division (mitosis) is the process by which identical copies of the replicated genome are segregated into two identical daughter cells. During this procedure, mitotic motors play important roles in regulation of cell division. Uncontrolled cell division causes cancer. Understanding the mechanism of cell division and its regulation has thus become a key to finding cures for cancer. Cytokinesis is the last gate to control cell division. It is dominated by centralspindlin, a key motor complex consisting of Kinesin-6 (Pav) and RacGAP (Tum). However, little is known about the structure and function of centralspindlin. This is mainly because there are technical difficulties of expressing full-length centralspindlin. Here we use baculovirus expression system to express full-length centralspindlin motor complex for *in vitro* analysis.

Objective: Our goal for the project is to establish a baculovirus expression system to purify full-length centralspindlin, and perform *in vitro* assays to further understand its structure and function.

Methods: Sf9 cells from the species *Spodoptera frugiperda* were used in conjunction with Baculovirus to express motor proteins for structural and functional analysis. Two types of recombinant baculoviruses bearing the genes of Pav and Tum were generated. Through coinfection of Sf9 cells with these two viruses, the centralspindlin motor complex was expressed and purified for future structural analysis.

Conclusion: We have determined that baculovirus, in conjunction with Sf9, to be an effective tool for expression of full-length centralspindlin. Currently, we are working on the purification of the motor complex using Ni-NTA affinity chromatography. For future research, we will use electron microscopy and *in vitro* biochemical assays for further structural analysis.