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Comparing the Mean Scores of 10% Giemsa Staining Using Different Diluents for Malaria Diagnosis

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ABSTRACT

Malaria is a mosquito-borne disease caused by protozoans of the *Plasmodium* genus and transmitted through the bites of infected female Anopheles mosquitoes. It is endemic in tropical and subtropical regions, with the primary human-infecting species include P. falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi. Blood film staining using Giemsa diluted with phosphate buffer is the gold standard for malaria diagnosis. However, preparing phosphate buffer in the field is difficult and time consuming. This study aimed to identify a more affordable and easily accessible alternative diluent. Positive malaria slides stored at -30°C were stained using 10% Giemsa prepared using phosphate buffer, tap water or drinking water (20 slides per group). The staining results were evaluated by two screeners, and mean scores were recorded based on a scale of 1 (poor) and 2 (good). The study found mean score for 10% Giemsa diluted with phosphate buffer was the highest (2.0) compared to tap water (1.95) and drinking water (1.50). There was no significant difference between 10% Giemsa diluted with phosphate buffer and tap water (p=0.152), whereas there was a significant difference between 10% Giemsa diluted with phosphate buffer and drinking water (p< 0.001). The pH of tap water (7.0) was within the ideal range for a diluent (7.0–7.4), while pH of drinking water (6.5) was not within this range, resulting in a lower mean score. In conclusion, this study found tap water can be used as an alternative diluent to phosphate buffer for Giemsa staining in malaria diagnosis.

Keywords: Diluent solution; drinking water; Giemsa staining; malaria; phosphate buffer; tap water

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Silent Spillover? Emerging Risks of Zoonotic Malaria Caused by *Plasmodium cynomolgi*

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ABSTRACT

The emergence of *Plasmodium cynomolgi* as a cause of sporadic malaria cases in Southeast Asia has raised increasing concern regarding its zoonotic potential. Although traditionally recognised as a non-human primate (NHP) parasite, recent reports of natural human infections suggest that it may represent an underestimated public health threat. To better understand its epidemiology and transmission dynamics, an extensive literature search was conducted using PubMed, Scopus, Web of Science, Google Scholar, and other relevant databases to identify studies published from the 1960s to May 2024. From a total of 5,497 retrieved articles, 106 were selected for inclusion based on relevance. Analysis of these studies indicates that P. cynomolgi demonstrates higher prevalence in both macaque and mosquito populations compared to Plasmodium knowlesi, the most frequently reported zoonotic malaria parasite in the region. A significant challenge arises from the close morphological resemblance of P. cynomolgi to Plasmodium vivax, which often results in misdiagnosis and underreporting, thereby masking its true distribution. In addition, experimental studies in vitro and in vivo confirm its ability to invade human reticulocytes through mechanisms analogous to those of P. vivax, reinforcing its potential to establish infections in human hosts. These findings highlight significant gaps in current diagnostic and surveillance systems, especially in endemic settings where circulation of the parasite may be overlooked. Overall, this review emphasises the urgent need for sensitive molecular diagnostic tools, enhanced surveillance, and targeted vector control strategies to mitigate the risk of *P. cynomolgi* emerging as a major zoonotic malaria in future.

Keywords: Anopheles; malaria; non-human primate (NHP); Plasmodium cynomolgi; zoonotic

Evaluating the Anti-amoebic Effect of Silver Nanoparticles Synthesised Using *Citrus aurantifolia* Fruit Peel Extract Against *Acanthamoeba* Keratitis

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ABSTRACT

Acanthamoeba keratitis is a serious corneal infection caused by the common protozoan Acanthamoeba, frequently linked to poor contact lens hygiene. Recently, due to a decrease in the efficacy of some disinfecting solutions, there has been a rising interest in alternative disinfection methods with nanoparticles emerging as a promising option for anti-amoebic agents. This study investigated the anti-amoebic effects of silver nanoparticles (AgNPs) synthesised using Citrus aurantifolia fruit peel extract (CAFPE) on Acanthamoeba trophozoites. The silver nanoparticles were biogenically formulated using CAFPE, an eco-friendly and cost-effective method that serves as a reducing, stabilising, and capping agent. Preliminary screening with UV-vis confirmed a complete synthesis of AgNPs at an absorption peak of 440 nm, while TEM and SEM analysis revealed evenly distributed smooth and spherical-shaped AgNPs with an average size of 15.15645 nm. Further characterisation with FTIR, DLS, EDX, and XRD also depicted the formulation of AgNPs with enhanced surface plasmon resonance properties. The amoebicidal assay was performed in vitro by exposing the Acanthamoeba castellanii trophozoites to various concentrations of the AgNPs in a dose-dependent manner. At the same time, the trypan blue exclusion method was used to measure the viability of cells after treatment. Treating Acanthamoeba trophozoites with the synthesised CAFPE-AgNPs showed a significant reduction in the viability of the trophozoites compared to the controls (p<0.05). Thus, silver nanoparticles synthesised using CAFPE demonstrated strong antiamoebic effects against Acanthamoeba trophozoites in vitro and represent a potential agent for the prevention of Acanthamoeba keratitis.

Keywords: *Acanthamoeba* keratitis; anti-amoebic activity; biogenic synthesis; *Citrus aurantifolia* fruit peel extract (CAFPE); silver nanoparticles

Overview of *Entamoeba histolytica* Intestinal Infection in Malaysia

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ABSTRACT

Entamoeba histolytica (E. histolytica) is a protozoan parasite that causes intestinal amoebiasis in humans. The infection remains a health concern in developing countries where sanitation and water quality are poor. In Malaysia, several studies have reported the occurrence of E. histolytica, but a clear national picture of its prevalence is limited. This review summarises published data on human intestinal E. histolytica infection in Malaysia and provides an overall estimate of its occurrence. Fourteen studies published up to 2025 were included, covering different population groups and states across the country. The combined prevalence of E. histolytica infection was 7%, with substantial variation among studies. The highest prevalence was recorded in Pahang (18%) and Perak (10%), while Kelantan (2%) showed the lowest. In subgroup analysis, aboriginal schoolchildren had the highest infection rate (16%), whereas urban residents and patients with gastrointestinal symptoms showed the lowest (2%). Studies based on microscopy reported higher prevalence (7%) than those using molecular methods (4%), indicating possible misidentification of non-pathogenic species. Overall, the findings suggest that although E. histolytica infection is not widespread in Malaysia, continued monitoring and improved diagnostic approaches are needed, especially among rural and indigenous communities.

Keywords: Entamoeba histolytica; intestinal amoebiasis; Malaysia; prevalence; review

Effects of Medicinal Plants on Blastocystis Infection

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ABSTRACT

Blastocystis spp. is a common intestinal protozoan in humans, though its pathogenic role remains uncertain. The standard treatment, metronidazole, often shows variable effectiveness, and emerging resistance has prompted interest in natural alternatives. This review summarises studies that examined the effects of medicinal plants and their bioactive compounds against Blastocystis infection in laboratory and animal models. A total of 27 studies were analysed, most of which were conducted in vitro. Commonly tested plants, such as garlic and ginger, showed strong inhibitory activity, supported by limited in vivo evidence demonstrating reduced cyst shedding and inflammation. Several plant extracts exhibited comparable or greater activity than metronidazole. Overall, the findings indicate that medicinal plants hold potential as alternative or supportive therapies for Blastocystis infection, particularly in cases of drug resistance. Further clinical studies are essential to evaluate their safety and therapeutic value in humans.

Keywords: Alternative therapy; Blastocystis spp.; medicinal plants; metronidazole resistance; review

PI-06

Pongamol as a Potential Adenosine Deaminase (ADA) Inhibitor for the Treatment of *Blastocystis hominis* Infection

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ABSTRACT

Targeted treatment for *Blastocystis hominis*, a prevalent gut parasite associated with immunological and gastrointestinal disorders, remains limited. One potential strategy is to inhibit adenosine deaminase (ADA), a key enzyme in purine metabolism and immune regulation. Pongamol, a plantderived molecule, was investigated for its ability to modulate immunological responses and prevent parasite persistence. Among several host-related proteins, Pongamol demonstrated the highest binding affinity to ADA (-8.7 kcal/mol) in molecular docking studies. Stable binding, mediated through hydrogen bonds and zinc ion coordination, was validated by LigPlot analysis. Evaluation using SWISS ADME indicated favourable drug-like characteristics, such as high gastrointestinal absorption and adhered to the Lipinski's Rule of Five. Pongamol may also affect gut-brain axis responses, as evidenced by its capacity to pass the blood-brain barrier. STITCH network analysis highlighted ADA's role in immunological modulation, suggesting that Pongamol-mediated inhibition may help control inflammation, a common feature of Blastocystis infections. Toxicity profiling indicated no mutagenic or carcinogenic concerns, though moderate hepatotoxicity and reproductive risks were noted. Overall, Pongamol shows promise as an oral therapeutic candidate for B. hominis infection by inhibiting ADA and modulating host immune responses. Further in vivo and in vitro studies are warranted to confirm its efficacy and safety, particularly in vulnerable populations.

Keywords: Adenosine deaminase (ADA); *Blastocystis hominis*; immune modulation; molecular docking; Pongamol

Epidemiology of Human Blastocystis spp. in Malaysia

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ABSTRACT

Blastocystis spp. is a unicellular intestinal protozoan parasite with a debated role in human disease. It is frequently reported in developing regions, particularly among immunocompromised individuals and those with frequent animal contact. This study summarises prevalence and genetic distribution of Blastocystis spp. in Malaysia. We included cross-sectional and hospital-based studies that examined different populations in Malaysia. These populations encompassed urban and rural communities, immunocompromised patients (such as those undergoing chemotherapy or living with HIV), individuals with comorbidities (e.g., cancer, gastrointestinal disorders), and high-risk groups with frequent animal exposure, including Orang Asli communities and zoo or farm workers. The parasite was detected in nine states, with an overall infection rate of 17.8% (1671/9397). Prevalence was highest in Pahang (27.3%) and lowest in Johor (3.4%). Molecular analyses identified six subtypes (ST1-ST6), with ST3 as the dominant subtype across all states (54.7%, 338/618). The predominance of ST3 highlights its potential adaptation to the Malaysian population and environment. This study also points to gaps in research from East Malaysia and limited data on subtype-symptom associations. Future studies with larger sample sizes and standardised diagnostic methods are warranted to improve comparability and strengthen epidemiological evidence. Overall, these findings enhance current understanding of Blastocystis epidemiology in Malaysia and provide useful insights to guide national health policies and control strategies against this parasite.

Keywords: Blastocystis spp.; Malaysia; meta-analysis; subtype distribution

PI -08

Evaluation of the Larvicidal Effects of Acetamiprid and Imidacloprid on *Aedes aegypti* Larvae

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ABSTRACT

Dengue remains a public health challenge in Malaysia, despite extensive usage of chemical insecticide as vector control. Neonicotinoids such as acetamiprid and imidacloprid target insect nicotinic acetylcholine receptors (nAChRs), yet their larvicidal efficacy and potential for resistance development in local mosquito populations remain poorly studied. This study investigates the susceptibility status of neonicotinoids against Aedes aegypti larvae from urban areas in Kuala Lumpur and Selangor. Eggs from various localities were collected from the field, hatched and reared in the laboratory. Larvicidal bioassays were conducted on field strains and Ae. aegypti Bora-Bora laboratory strains to determine lethal concentration 50 (LC₅₀) values for acetamiprid and imidacloprid using probit analysis in IBM SPSS Statistics version 26. Six replicates of 20 larvae per concentration were tested. Concentration ranges were 50-150 µg/L (acetamiprid) and 30-100 µg/L (imidacloprid) for the laboratory strain, and 100-1000 μg/L (acetamiprid) and 75-750 μg/L (imidacloprid) for field strains. One-way ANOVA showed significant differences in LC₅₀ values among the strains for both acetamiprid (F(10,55)=1305, p<0.0001) and imidacloprid (F(10,55)=1566, p<0.0001). Tukey's post hoc test indicated that the laboratory strain was significantly different from all field strains. Overall, most field strains were significantly different from each other, except for certain pairs that showed no differences. Resistance ratios remained <5 across all sites, confirming susceptibility to both compounds. Aedes aegypti in Kuala Lumpur and Selangor are susceptible to acetamiprid and imidacloprid. Therefore, these insecticides could be incorporated into chemical rotation strategies, thereby supporting dengue vector control programs.

Keywords: Aedes aegypti; acetamiprid; imidacloprid; larvicide; mosquito larvae; neonicotinoid

Revealing the Microbial Composition of the Plaster Bagworm (*Phereoeca* sp.): Potential Links to Inflammation and Human Health

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ABSTRACT

The Household Casebearer, Phereoeca uterella, commonly referred to as the Plaster Bagworm and known locally in Malaysia as 'Kamitetep' is categorised as a pest that damages textiles and may pose risks to human health. In Malaysia, anecdotal reports suggest that contact with *Phereoeca* sp. may be associated with skin rashes and inflammation, but this remains poorly understood and understudied. Therefore, this study aimed to (i) investigate the microbial composition of the species and (ii) identify pathogenic microbes that could potentially cause inflammation and itchiness. Next Generation Sequencing (NGS) targeting 16S ribosomal RNA (rRNA) was performed on nine individuals using metabarcoding analysis via the Illumina MiSeq platform. The dominant bacterial phyla identified were Proteobacteria and Actinobacteriota (total 72%), while the most prevalent classes included Actinobacteria and Alphaproteobacteria and Gammaproteobacteria (total 72%). Notably, the families Pseudonocardiaceae, Beijerinckiaceae, and Burkholderiaceae were prominent, with Enterobacteriaceae being of particular interest due to their potential implications for human health. Several genera were detected within Phereoeca sp., including Bacillus, Enterobacter, Pseudomonas, Mycobacterium, Pseudonocardia, and Staphylococcus, some of them known to be associated with inflammation and histamine production. These findings provide a foundation for future research into the medical relevance of *Phereoeca* sp. and its potential impact on human health.

Keywords: Histamine; metagenomic; microbiomes; next generation sequencing; plaster bagworm

PI-10

Assessing the Effectiveness of the Digital Thermo Mosquito Blood Feeder (DITMOF) for Artificial Feeding of Aedes albopictus

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ABSTRACT

Digital Thermo Mosquito Blood Feeder (DITMOF) is an in-house device designed for the artificial blood feeding of mosquitoes. It has been proven effective for Aedes aegypti, the primary vector for dengue fever. However, its effectiveness has not yet been evaluated for Aedes albopictus, another important vector of dengue and other mosquito-borne diseases. Therefore, this study aims to compare the effectiveness of bovine blood heated using DITMOF and direct feeding on mice (DF) (as control), based on the following parameters: blood feeding rate, average egg produced (fecundity rate), egg hatching rate and female survival rate in a laboratory strain of Ae. albopictus. For each feeding method, 20 female mosquitoes aged 3 to 5 days were placed in a 30 x 30 x 30 cm cage. One group was offered bovine blood using DITMOF+PTFE tape, while the other group was offered a live white mouse (Mus musculus). The feeding period lasted for 30 minutes, and six replicates of experiments (n=120 mosquitoes per method) were performed for both feeding methods. The feeding rate with DITMOF (80% \pm 0.9) was significantly higher than with the DF (72.5% \pm 0.4) (p<0.05). While fecundity and egg hatching rates did not differ significantly between methods (p=0.23, p=0.44), the DITMOF group averaged 10 eggs per female versus 8 eggs from direct feeding. Hatching rates were similar at 74.8% and 76.6%, respectively. Female survival 7 days post-feeding was higher in the DITMOF group (93%) than the DF group (86%). This supports DITMOF's use for Ae. albopictus laboratory colonisation.

Keywords: *Aedes albopictus*; artificial feeding; bovine blood; dengue; Digital Thermo Mosquito Blood Feeder (DITMOF); direct feeding

PI -11

Effect of Mortuary Chamber on the Development of Forensically Important Blow Fly Larvae *Chrysomya megacephala* and *Chrysomya rufifacies*: Implications for Postmortem Interval Estimation

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ABSTRACT

The growth and developmental data of forensically important blow flies play a crucial role in estimating the postmortem interval (PMI). However, for larval samples collected during autopsy, analysis of developmental rates without accounting for the temperature disparity between the low-temperature mortuary chamber and the high-temperature larval mass may lead to inaccurate PMI estimations. This study compares the effects of mortuary chamber on the development of Chrysomya megacephala (Fabricius) (Calliphoridae) and Ch. rufifacies (Macquart) (Calliphoridae) larvae collected from bodies at the scene of death and during autopsy. A total of 12 cadaver cases infested with forensically important blow flies and referred to Hospital Canselor Tuanku Muhriz were examined. Parameters assessed include larval survivability, larval length and duration of development to adulthood. Results showed that larval/pupal samples collected at the death scene exhibited a significantly higher adult emergence rate (p<0.001 for Ch. megacephala and p<0.05 for Ch. rufifacies) and a shorter mean developmental period (p<0.001) compared to those collected during autopsy. Further analysis also revealed a significant difference (p<0.001) between the minimum and mean developmental durations in both sample groups. In conclusion, failure to consider the effect of mortuary chamber may lead to underestimation of the PMI. Thus, entomological evidence should ideally be collected at the death scene; while collection during autopsy remains viable, it necessitates consideration of a broader PMI range.

Keywords: *Chrysomya megacephala*; *Chrysomya rufifacies*; mortuary chamber; postmortem interval; PMI;

PI-12

Advancing Lymphatic Filariasis Diagnostic: Automated Detection and Quantification of Microfilariae in Blood Smears Using YOLOv10

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ABSTRACT

Lymphatic filariasis is a neglected tropical disease affecting over 657 million people globally, primarily in tropical and subtropical regions. The traditional diagnostic method, which relies on manual microscopic examination, is time-consuming, dependent on expert skill, and challenging, especially when a large volume of samples is involved. This study aims to develop an automated framework for detecting and quantifying microfilariae in Giemsa-stained thick blood smears using the YOLOv10 object detection model. The system was trained on a curated dataset of 3,000 blood smear images from the Institute of Public Health (IKU), preprocessed and annotated in VOC format. YOLOv10's architecture was enhanced using lightweight convolutional structures, large kernel filters, and BiFPN feature fusion to improve small object detection accuracy. Experimental evaluation demonstrated strong model performance with an accuracy of 84.8%, precision of 86.6%, recall of 97.6%, F1-score of 0.918, and mAP@0.5-0.95 of 66.7%. The model consistently performed well across both clean (easy) and complex (difficult) blood smear images, maintaining high sensitivity and robustness in diverse visual environments. Comparative analysis with DeepLabv3 showed that YOLOv10 outperformed it significantly in mIoU (66.7% vs. 50%) and mean pixel accuracy (94% vs. 50%), while achieving faster inference speed. This study highlights the potential of YOLOv10 for real-time microfilariae detection in resource-limited or high-throughput settings. Future efforts will aim to enhance model interpretability, streamline deployment, and support clinical integration for intelligent public health diagnostics.

Keywords: Deep learning; lymphatic filariasis; medical image analysis; microfilariae detection; public health diagnostics

Strongyloidiasis Mimicking Lymphatic Filariasis: A Case of Hypereosinophilia with Scrotal Involvement

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ABSTRACT

Strongyloidiasis, caused by Strongyloides stercoralis, is often underdiagnosed due to its nonspecific clinical manifestations and the low sensitivity of stool microscopy. It may also be misdiagnosed. This report aimed to highlight the diagnostic challenges of strongyloidiasis presenting with atypical features resembling lymphatic filariasis. A 60-year-old lorry driver was hospitalised with persistent fever, diarrhoea, eosinophilia (1,100/µL), and left testicular pain. Ultrasonography revealed minimal bilateral hydrocoeles with echogenic sediments, a bulky epididymis, and a prominent pampiniform plexus suggestive of bilateral varicocele. Based on the clinical presentations, lymphatic filariasis was initially suspected, and the patient's serum was tested with the Reszon Filariasis IgG4 rapid test. Subsequently, based on the patient's occupational exposure to areas contaminated with dog faeces, Strongyloides IgG4 rapid test, stool microscopy, and real-time PCR of the stool were performed. The rapid test results were negative for lymphatic filariasis, but positive for Strongyloides. The stool examination was negative for Strongyloides larvae, but the Strongyloides PCR test was positive. The patient initially received empirical antifilarial therapy (albendazole, ivermectin, diethylcarbamazine) based on the symptoms, but following the laboratory results, he was treated for strongyloidiasis. His fever resolved, eosinophilia normalised and scrotal symptoms improved. This report demonstrates that S. stercoralis infection can mimic lymphatic filariasis, presenting with hypereosinophilic syndrome and scrotal involvement. It also highlights the limitations of stool microscopy and emphasises the importance of incorporating serological and molecular tools to enhance diagnostic accuracy, improve clinical recognition, ensure timely management, and reduce inappropriate therapy.

Keywords: Hypereosinophilia; lymphatic filariasis; scrotal involvement; *Strongyloides stercoralis*; strongyloidiasis

Identification and Production of *Enterocytozoon bieneusi*Protein (B7XJ00) Diagnostic Marker for Disseminated Microsporidiosis

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ABSTRACT

Enterocytozoon bieneusi is a major cause of intestinal microsporidiosis. The disseminated infections are often underreported in immunocompromised patients due to non-specific symptoms. A hypothetical E. bieneusi protein (B7XJ00) was found in the blood of HIV/AIDS patients. However, E. bieneusi cannot be cultured, and B7XJ00's function remains largely unknown. Encephalitozoon cuniculi is a suitable surrogate because of high genetic homology with E. bieneusi. This study aimed to assess the antigenicity of recombinant B7XJ00 protein (rB7XJ00) against plasma antibodies from disseminated microsporidiosis patients and identify the E. cuniculi proteins reactive to anti-rB7XJ00 polyclonal antibodies (anti-rB7XJ00 pAb). The rB7XJ00 protein was produced, purified, and validated using anti-His-tag Western blot. The antigenicity of rB7XJ00 was evaluated using plasma from 23 microsporidiosis patients and 22 healthy individuals. New Zealand white rabbits were immunised to produce anti-rB7XJ00 pAb. E. cuniculi lysate was tested with anti-rB7XJ00 pAb, and reactive E. cuniculi proteins were analysed by mass spectrometry to identify B7XJ00's function. Western blot detected three protein bands (10, 25 and 75 kDa), confirmed as E. bieneusi by LCMS/MS. B7XJ00 showed 65.2% (15/23) sensitivity and 77.3% (17/22) specificity when tested using plasma of microsporidiosis patients and healthy individuals. The reactive E. cuniculi protein was identified as mitochondrial heat shock protein 70 OS Encephalitozoon cuniculi (strain GB-M1). Its nuclear

localisation, similar to the predicted location of B7XJ00, suggests a functional resemblance between the two proteins. In conclusion, rB7XJ00 shows potential as a diagnostic marker for microsporidiosis, paving the way for developing more effective diagnostic tools.

Keywords: Circulating antigens; disseminated microsporidiosis; *Enterocytozoon bieneusi*; immunocompromised patients; recombinant protein

PI-15

Cutaneous and Nasopharyngeal Talaromycosis with Cranial Nerve Palsies: A Rare Nasopharyngeal Carcinoma Mimic in A Newly Diagnosed HIV

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ABSTRACT

Talaromyces marneffei is a thermally dimorphic fungus responsible for localised or disseminated talaromycosis. It primarily affects individuals with advanced HIV infection. This case report describes unexpected talaromycosis in a patient presenting with an unusual nasopharyngeal mass with multiple cranial nerve palsies. A 54-year-old man with preexisting bilateral chronic otitis media with mastoiditis presented with progressive dysphagia, hoarseness of voice, and nasal discharge for two weeks. The physical examination revealed a cachectic physique with several painless umbilicated papules on the face and neck. A nasoendoscopy revealed a nasopharyngeal mass. A contrasted CT scan of the head and neck revealed soft tissue thickening in the nasopharynx and oropharynx with cervical lymphadenopathy, suggestive of a nasopharyngeal tumour. Histopathological examination of the mass showed no malignancy but identified scattered yeast-like cells, and culture confirmed the presence of T. marneffei. HIV serology was positive, with a CD4 count of 76 cells/mm³ and a viral load of 4,720,000 copies/mL. An intravenous amphotericin B deoxycholate 0.7 mg/kg/day was initiated for nasopharyngeal talaromycosis. Nasopharyngeal talaromycosis presented as a mass is a very rare manifestation. In contrast to mucormycosis, cranial nerve involvement is not a distinguishing feature of talaromycosis. Amphotericin B deoxycholate remains the initial effective antifungal therapy for severe T. marneffei infection. In an HIV-positive patient with a nasopharyngeal mass, a fungal infection should thus be considered. An appropriate treatment is essential to avert fatalities from talaromycosis.

Keywords: Acquired immunodeficiency syndrome; amphotericin B; HIV; nasopharyngeal carcinoma; talaromycosis

PI-16

COVID-19 Vaccination and Dengue Fever Outcomes: Implications for Risk Management and Health Policy

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ABSTRACT

Emerging evidence suggests immune modulation from COVID-19 vaccination may influence the clinical course of other viral infections, including dengue. Understanding this interaction is crucial in regions where both viruses co-circulate. Nevertheless, the impact of COVID-19 vaccination on the severity of dengue fever complications remains unclear. This study investigates the relationship between COVID-19 vaccination and dengue severity, with a focus on hematological complications. A total of 168 confirmed dengue cases vaccinated against COVID-19 between September 2021 and July 2022 were studied. Hematological parameters assessed included platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT), D-Dimer, red blood cell (RBC) count, and the white blood cell (WBC) count. Among these cases, 108 had platelet count below 100,000/ mL; 30 showed prolonged PT (>20s), and 30 had elevated APTT (>40s). Logistic regression analysis identified PT (odd ratio [OR]: 2.19, p<0.001), APTT (OR: 0.69, p<0.001) and WBC count (OR: 1.18, p=0.004) as significant factors influencing dengue. Furthermore, the receiver operating characteristic (ROC) showed that PT had the highest diagnostic value (area under the curve [AUC]: 0.89), while D-Dimer and WBC had moderate value (AUC: 0.59 and 0.58, respectively). APTT demonstrated low diagnostic power (AUC: 0.07). These findings suggest that PT, APTT, and WBC counts may help assess dengue severity in COVID-19-vaccinated individuals. Although D-Dimer lack specificity, it could serve as a sensitive screening indicator. The information may aid in improved risk stratification and clinical care of dengue in vaccinated populations.

Keywords: COVID-19 vaccination; coagulation markers; dengue severity; post-vaccination complications; prothrombin time

PI -17

Mechanisms of Probiotics and Synbiotics for Enhancing Human Health

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ABSTRACT

Probiotics play a pivotal role in maintaining host health by shaping microbial interactions and supporting symbiotic relationships between humans and their microbiomes. This study highlights their diverse applications, ranging from enhancing immunity, supporting growth, and preventing diseases to stabilising gut microbial communities. Advances in molecular and genomic techniques have enabled the customisation of probiotics, with species such as *Enterococcus, Bifidobacterium*, and *Lactobacillus* showing promising therapeutic potential. We further discuss the synergistic effects of synbiotics, which enhance probiotic survival and functionality, amplifying their beneficial impact. Probiotics also contribute to disease prevention and therapy through mechanisms such as immune modulation, reduction of oxidative stress, and production of bioactive metabolites like bacteriocins and exopolysaccharides. These compounds play an essential role in gut health and may offer protective effects against gastrointestinal disorders. Together, these insights emphasise the wideranging potential of probiotics and synbiotics as tools for improving human well-being.

Keywords: Gut microbiota; microbial interactions; probiotics; secondary metabolites; synbiotics

Haemophagocytic Lymphohistiocytosis in *Plasmodium* falciparum Malaria: A Case Report

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ABSTRACT

Haemophagocytic lymphohistiocytosis (HLH) is a rare but life-threatening hyperinflammatory syndrome often triggered by infections, malignancy, or autoimmune diseases. Malaria-induced HLH is uncommon and typically associated with severe disease; its occurrence following uncomplicated malaria poses a significant diagnostic challenge. This case describes a 22-year-old previously healthy Burmese male presented with fever and malaise after working 3 months in a malaria-endemic area in Malaysia. He was diagnosed with uncomplicated Plasmodium falciparum malaria and treated with artemether-lumefantrine and primaquine. Despite parasitaemia clearance, he developed persistent high-grade fever, severe transaminitis, and hepatosplenomegaly. Laboratory findings revealed thrombocytopenia, hyperferritinaemia and hypertriglyceridaemia. Bone marrow biopsy confirmed haemophagocytosis, fulfilling six of the eight HLH-2004 diagnostic criteria. The patient was diagnosed with secondary HLH and started on intravenous dexamethasone, resulting in rapid clinical and biochemical improvement. He was discharged with an 8-week steroid tapering dose and outpatient follow-up. This case is notable for the development of HLH following uncomplicated Plasmodium falciparum infection, a rare clinical scenario. Clinical features such as fever, cytopenias, and organomegaly may mimic severe malaria, making HLH diagnosis particularly challenging. To our knowledge, this is the first documented case of malaria-associated HLH in Malaysia. HLH should be considered in patients with ongoing systemic inflammation after malaria treatment, even in the absence of severe parasitaemia. Early recognition and immunosuppressive therapy are critical to improve patient outcomes.

Keywords: Haemophagocytic lymphohistiocytosis (HLH); haemophagocytosis; malaria; *Plasmodium falciparum*

Exploring Native Zingiberaceous Wonders in the Battle Against Brain-Eating Amoeba: A Transcriptome-Based Approach to Bioactive Compound Discovery

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ABSTRACT

Primary amoebic meningoencephalitis (PAM), caused by *Naegleria fowleri* (*N. fowleri*), is a rare but rapidly fatal infection with limited therapeutic options. Essential oils (EOs) derived from the Zingiberaceae family have demonstrated promising anti-amoebic activity. We hydrodistilled EOs from Common Ginger (*Zingiber officinale*), Red Ginger (*Z. officinale var. rubrum*), and Bentong Ginger (*Z. officinale cv. Bentong*), and profiled their chemical composition using gas chromatography–mass spectrometry (GC-MS) and gas chromatography–flame ionisation detection (GC-FID). Amoebicidal potency was assessed via Trypan blue exclusion. *N. fowleri* trophozoites used in this study were obtained from the American Type Culture Collection (ATCC 30174). Bentong Ginger EO exhibited the highest cidal efficacy at 75 μ M, while Red and Common Ginger EOs showed moderate to minimal activity. Host-cell safety was confirmed by LDH assays in HaCaT keratinocytes and SH-SY5Y neuroblastoma cells, indicating negligible cytotoxicity at active concentrations. These findings underscore Bentong Ginger EO as a potent natural candidate against *N. fowleri* and support further preclinical development for PAM therapy.

Keywords: Anti-amoebic activity; ginger essential oils; Naegleria fowleri; programmed cell death

Prompt Diagnosis and Treatment of Acanthamoebic Keratitis in a Contact Lens User: A Case Report

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ABSTRACT

We present a case of successfully treated acanthamoebic keratitis (AK) in a contact lens user, focusing on key clinical features and microscopic findings. A 34-year-old Malay woman, a kindergarten teacher, presented with right eye redness, severe pain, and blurred vision for four days. She is a daily contact lens user. The pain was disproportionately severe compared to the degree of conjunctival injection. She was initially diagnosed with corneal abrasion and treated with topical antibiotics and lubricants, but symptoms persisted and worsened after one week, prompting referral to our centre. Visual acuity in the right eye was 6/24 (pinhole 6/12). On examination, there was lid swelling, injected conjunctiva, a faint linear whitish stromal infiltrate centrally with radial perineural infiltrates at 10 o'clock extending until the paracentral cornea. The epithelium was intact but slightly irregular, with fine endothelial dusting. Given the contact lens history, severe pain, and presence of radial perineuritis, a clinical diagnosis of early AK was made. Corneal culture later confirmed Acanthamoeba cysts and trophozoites. Intensive topical polyhexamethylene biguanide (PHMB), chlorhexidine and fortified antibiotics were commenced. After two weeks, pain and redness improved significantly, the stromal infiltrates became less dense, and visual acuity improved to 6/9. The patient remained on a tapering anti-amoebic regimen with close follow-up to monitor for recurrence. Early recognition of radial perineuritis and disproportionate pain in contact lens wearers is critical for the clinical diagnosis of AK. Prompt initiation of targeted therapy can improve outcomes and preserve vision.

Keywords: Acanthamoebic keratitis; antimicrobial therapy; contact lens; corneal infiltrates

Trichomonas tenax in Periodontitis: A Review of Prevalence, Risk Factors and Detection Methods

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ABSTRACT

Periodontitis is a chronic inflammatory condition affecting the supporting structures of the teeth and is widely recognised as a significant public health concern. Trichomonas tenax, an oral protozoan residing in the oral cavity, has been frequently observed in periodontitis patients, yet its role in disease progression remains unclear. This literature review aimed to examine the prevalence of T. tenax, associated risk factors, and current detection methods. Relevant studies were identified from major databases including PubMed, Scopus, Web of Science and Google Scholar, with nine studies meeting the inclusion criteria for this review. The findings indicate that *T. tenax* is more prevalent in periodontitis patients than in healthy individuals, with the colonisation rate increasing proportionally with disease severity. Identified risk factors include poor oral hygiene, smoking, advanced age and systemic conditions such as diabetes. Detection of *T. tenax* was carried out using microscopy, culture, and molecular techniques, with PCR demonstrating superior sensitivity and specificity. Dental plaque was the most frequently used and effective sample type for its detection. Despite evidence suggesting a link between T. tenax and periodontitis, its exact pathogenic role remains underexplored. Further research is necessary to determine its contribution to periodontal inflammation and tissue destruction. This review contributes to the current understanding of *T. tenax* and highlights the need for its consideration in the diagnosis and management of periodontitis.

Keywords: Gum disease; oral parasite; oral protozoan; periodontitis; Trichomonas tenax

Amebic Liver Abscess with Pleuropulmonary Complications in a Young Adult Male

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ABSTRACT

Amebic liver abscess (ALA) is the most common extraintestinal manifestation of amebiasis caused by *Entamoeba histolytica*. We report a case of a 26-year-old Malaysian male patient with pain in the right lower quadrant who was initially diagnosed with acute appendicitis. Following laparoscopic appendicectomy, the recurrent right lower quadrant pain persisted, and it led to further investigations and the revelation of multiple well-defined hypodense lesions in segments V, VI, VII, and VIII of liver without any internal air locules, septation, or calcification in the imaging was consistent with amebic liver abscess. Diagnosis was confirmed using serology tests with positive *Entamoeba histolytica* serum IgG 3.143 OD. The patient was successfully treated with percutaneous drainage and metronidazole but developed a pleuropulmonary complication that was successfully managed. This case highlights the importance of a high suspicion of ALA in patients from amoebiasis endemic areas with a similar presentation, particularly among young men with a relevant travel history. It also demonstrates the risk of severe ALA complications, such as pleural involvement. Early diagnosis, good imaging, and quick medical intervention with luminal and tissue anti-agents are the key to the best outcomes.

Keywords: *Entamoeba histolytica*; metronidazole; percutaneous drainage; pulmonary; travelassociated risk

Optimising Fixatives and Stains for Preserving Trichomonas vaginalis Morphology: A Comparative Study

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ABSTRACT

Trichomonas vaginalis is the causative agent of trichomoniasis, the most prevalent non-viral sexually transmitted disease worldwide. Beyond its direct clinical impact, trichomoniasis is also associated with serious conditions such as cancer and increased HIV transmission. Since T. vaginalis morphology is highly sensitive to environmental changes, the choice of fixative and stain plays a critical role in accurate microscopic identification. This study aimed to evaluate the effects of different fixatives and stains on the preservation and visualisation of T. vaginalis morphology, and to determine the most effective combination for diagnostic use. Eight fixatives were tested: 70% ethanol, 95% ethanol, 100% ethanol, 100% methanol, sodium acetate acetic acid-formalin (SAF), Schaudinn's solution, polyvinyl alcohol (PVA) with a zinc base, and PVA with a mercury chloride (HgCl₂) base. Seventytwo-hour-old cultures of T. vaginalis trophozoites were stained with both Giemsa and trichrome stains. Morphological assessment was performed using a standardised scoring system. Methanol and ethanol at 100% showed comparable efficacy in preserving the parasite morphology with both Giemsa (p=0.464) and trichrome (p=0.401) staining. Overall, Giemsa staining demonstrated superior performance compared to trichrome, showing significant differences with three out of four fixatives, including 100% methanol (p<0.05) and 100% ethanol (p<0.01). These results highlight the importance of selecting appropriate fixatives and stains to optimise the morphological preservation of *T. vaginalis*, thereby improving diagnostic accuracy in clinical parasitology.

Keywords: Diagnosis; fixatives; morphological preservation; Trichomonas vaginalis; stains

Assessing the Prevalence of *Blastocystis* spp. among the Orang Asli Kensiu Community in Kedah, Malaysia

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ABSTRACT

Blastocystis spp. is one of the most common intestinal parasites worldwide. However, its distribution in Malaysia's indigenous populations is not well documented, especially in the Orang Asli population in the northern region of Peninsular Malaysia. This study investigated its prevalence among the Orang Asli Kensiu community of Kampung Lubuk Legong, Kedah. Stool samples were collected from 33 participants and processed within six hours of collection. Approximately 500 mg of each sample was cultured in Jones' medium enriched with 10% horse serum and incubated at 37°C for 72 hours, with daily microscopic examination carried out to detect vacuolar forms. The overall prevalence of Blastocystis was 45.5%, with infections detected in both children and adults. The relatively high percentage of infection suggests that exposure to the parasite is widespread within the community. The findings provide important baseline data on Blastocystis among the Kensiu tribe, highlighting the need for improved hygiene and sanitation measures to reduce transmission. By documenting the burden of infection in this underrepresented group, study contributes to a better understanding of the parasite epidemiology in Malaysia. It offers evidence to guide future public health strategies.

Keywords: Blastocystis spp.; in vitro cultivation; Kensiu community; Orang Asli; Peninsular Malaysia

Intestinal Parasitic Infections (IPIs) among Workers in Southeast Asia (SEA) Countries: A Narrative Review

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ABSTRACT

Intestinal parasitic infections (IPIs) remain a persistent global health challenge, disproportionately affecting vulnerable populations in developing countries. In Southeast Asia (SEA), these infections are not only a community health concern, but also pose occupational hazards to workers across various industries. This narrative review synthesises evidence from studies published between 2016 and 2025, identified through a comprehensive PRISMA-guided search, to examine the prevalence and determinants of IPIs among workers in SEA countries. Ten studies met the inclusion criteria. Reported prevalence varied widely, from 9.2% to 62.9%, reflecting differences in occupational settings, environmental conditions, and worker demographics. Across all studies, soil-transmitted helminths (Ascaris lumbricoides, Trichuris trichiura, hookworms) and intestinal protozoa (Entamoeba histolytica/dispar, Giardia sp. and Cryptosporidium spp.) were most frequently detected. Commonly reported risk factors included inadequate sanitation, poor personal hygiene, limited health literacy, and socioeconomic disadvantages, compounded by occupational exposures such as soil and animal contact, and handling of fecal matter. These findings underscore the persistence of IPIs in SEA's working populations and point to emerging occupational vulnerabilities. Targeted interventions, such as routine deworming, improved workplace and community sanitation, and enhanced health education, are critical in reducing the burden of IPIs and protecting workers' health.

Keywords: Intestinal parasitic infections; Southeast Asia; workers

Entomological Surveillance and Detection of Zoonotic Malaria Parasites Across Different Ecotypes in Gua Musang, Kelantan: Preliminary Findings

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ABSTRACT

Zoonotic malaria, caused by *Plasmodium knowlesi* and other non-human primate malaria parasites, is a growing public health concern in Southeast Asia, especially Malaysia. This ongoing study investigates the prevalence and infection status of Anopheles mosquitoes across different ecotypes to assess how anthropogenic land-use change influences their bionomics. Four locations in Gua Musang, Kelantan have been selected for the study with different ecotypes, including forest, forest fringes, village near a farm and village near a plantation. A total of 450 mosquitoes were collected from November 2024 to February 2025, comprising 116 Anopheles spp. and 334 from other genera. Of the Anopheles mosquitoes, 32 belonged to the Leucosphyrus group. The adult mosquitoes were collected using three different collection methods, including human landing catch (HLC) (n = 381), CDC light trap (n = 7) and Mosquito Magnet (n = 62). The collected mosquitoes were dissected and subjected to nested PCR to detect the presence of *Plasmodium* parasites. Overall, 3 An. introlatus were positive for Plasmodium parasites, which include P. inui (n = 1) and P. cynomolgi (n = 2). Preliminary analysis showed a higher abundance of Leucosphyrus group mosquitoes, known vectors of zoonotic malaria in villages near plantations and farms, suggesting that land-use changes may increase human-vector contact and sustain transmission risk. Further sampling and analysis are underway to refine infection rate estimates and assess seasonal variations. These interim findings underscore the importance of continuous vector surveillance in different ecotypes undergoing rapid change and provide an early warning framework for targeted vector control strategies.

Keywords: Knowlesi malaria; land use changes; Plasmodium sp.; zoonotic malaria

Replication Kinetics of Novel *Wolbachia* (*w*Cfe) in *Aedes albopictus*-derived Cell Line, C6/36

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ABSTRACT

Mosquito-borne arboviral diseases such as dengue and chikungunya have continuously been a major public health concern worldwide. Introducing intracellular bacterial endosymbiont Wolbachia in mosquito populations has proven to be an effective method in recent years to control the spread of arboviruses, most notably the dengue virus. Some Wolbachia strains have been propagated in insect cell lines, elucidating the advantages of cell cultures in providing a controlled environment for microbe-host interaction studies. The Wolbachia strain used in this study (wCfe) was a novel strain isolated from Malaysian Ctenocephalides felis fleas and subsequently maintained in an Ixodes scapularis-derived cell line, IDE8. To determine its replication kinetics in a new arthropod host cell line, wCfe was purified from IDE8 and inoculated into Aedes albopictus-derived cell line, C6/36. Real-time PCR targeting the Wolbachia 16S rRNA gene was used to quantify the extracted DNA from the infected culture. From day 1 post-infection (d.p.i.) to 4 d.p.i., a lag phase was observed, followed by exponential growth of the endosymbiont bacteria from 5 to 7 d.p.i. wCfe remained stably infected until the end of observation at 10 d.p.i., with an approximately 23 to 36.67-fold increase from day 1 post-infection. In all replicates, the generation time of wCfe in C6/36 was between 1.2 and 1.4 days. The findings suggest successful establishment of wCfe in C6/36 and substantial growth over the observation period. However, long-term observation is necessary to determine the persistence of wCfe in C6/36 over an extended period of time.

Keywords: Intracellular bacteria; mosquito cell line; real-time PCR; Wolbachia

Rare Manifestations of *Achromobacter xylosoxidans*: A Case Series of Pneumonia and Septic Arthritis

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ABSTRACT

Achromobacter xylosoxidans is an aerobic, oxidase-positive, non-fermenting Gram-negative bacillus widely distributed in the environment. It is an uncommon opportunistic nosocomial pathogen, notoriously known for its intrinsic resistance to various classes of antibiotics. This case series describes two distinct cases of multidrug-resistant (MDR) A. xylosoxidans pneumonia and joint infection. Case 1: A 39-year-old male with preexisting diabetes mellitus and hypertension was hospitalised for a right basal ganglia haemorrhage. During his hospitalisation, he developed a fever with signs of bilateral pulmonary infection. Bronchoalveolar lavage (BAL) culture yielded A. xylosoxidans, identified via matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry. Automated antibiotic susceptibility testing (AST) using the VITEK® 2 system showed MDR patterns susceptible only to ceftazidime and trimethoprim-sulfamethoxazole. The patient received intravenous trimethoprim-sulfamethoxazole, and subsequent chest X-rays indicated significant improvement. Repeated BAL cultures were negative. Case 2: A 68-year-old female with underlying diabetes mellitus underwent left knee arthrotomy washout for left knee septic arthritis. 10 mL of pus was drained, and the culture grew MDR A. xylosoxidans. Like Case 1, the isolate was susceptible to ceftazidime and trimethoprim-sulfamethoxazole. She was discharged with oral trimethoprim-sulfamethoxazole for a period of 6 weeks, and her condition improved remarkably. A good clinical response has been documented with trimethoprim-sulfamethoxazole, antipseudomonal penicillins (ceftazidime and piperacillin/tazobactam), and carbapenems. Risk factors for A. xylosoxidans infection include the use of medical devices, the presence of comorbidities and previous hospitalisation. Utilising molecular approaches for prompt identification and automated AST aids in directing therapy to attain both clinical and microbiological cures.

Keywords: Achromobacter sp.; infectious arthritis; multidrug resistance; pneumonia

Tracking Antibiotic Resistance and Serotype Trends in Non-Typhoidal Salmonella: A Two-Year Retrospective Study in Sultanah Aminah Hospital, Johor Bahru, Malaysia

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ABSTRACT

Non-typhoidal Salmonella (NTS) infections are a significant public health concern, particularly in regions with evolving antibiotic resistance. This study aimed to characterise serotype distribution, antimicrobial resistance (AMR), and multidrug resistance (MDR) trends among NTS isolates in Johor Bahru, Malaysia. A retrospective analysis of 262 Salmonella isolates (January 2023-December 2024) from Hospital Sultanah Aminah was conducted. Serotyping and antibiotic susceptibility testing (ampicillin, ceftriaxone, ciprofloxacin, trimethoprim-sulfamethoxazole) were performed. Statistical analyses assessed associations between serotypes, resistance patterns, and clinical outcomes. Salmonella Enteritidis (50.8%) dominated, followed by S. Weltevreden (20.6%). Resistance to ampicillin (24.5%) and ciprofloxacin (21.8%) was prevalent, with MDR observed in 4.2% of isolates. Invasive samples (blood, sterile fluids) were significantly associated with dominant serotypes ($\chi = 23.835$, p<0.001). Mortality correlated with invasive infections ($\chi = 20.575$, p<0.001) and nonsurgical departments (x = 3.986, p=0.046). Seasonal peaks occurred in mid-2024, aligning with global trends. High ampicillin resistance and emerging ciprofloxacin non-susceptibility highlight urgent stewardship needs. Southern Malaysia's AMR patterns mirror regional and global trends but underscore unique serotype dynamics. This study provides critical baseline data for targeted interventions in a high-burden setting.

Keywords: Antimicrobial resistance; Malaysia; multidrug resistance; Salmonella; serotypes

Natural Multi-Pathogen Inhibition by L001 *Lactobacillus plantarum* Extract: A Potential Biopreservative

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ABSTRACT

Foodborne pathogens such as Listeria monocytogenes, Salmonella typhi, Escherichia coli and Staphylococcus aureus remain major public health threats, while opportunistic bacteria like Klebsiella pneumoniae and Pseudomonas aeruginosa contribute to hospital-acquired infections and antimicrobial resistance. Although antibiotics are commonly prescribed, their overuse accelerates resistance, creating an urgent need for natural alternatives. This study evaluated the antimicrobial potential of a metabolite extract from Lactobacillus plantarum L001 against foodborne and nosocomial pathogens. Crude metabolites were extracted and tested using the broth microdilution method with optical density (OD600) measurements to assess growth inhibition. Heat-inactivated L001 was also evaluated, and its interaction with L. monocytogenes was visualised by scanning electron microscopy (SEM). L001 extract showed broad-spectrum inhibitory activity with a minimum inhibitory concentration (MIC) of 0.25 g/mL for all tested pathogens, with net OD₆₀₀ readings remaining below 0.01, confirming effective growth suppression. SEM revealed that L001 cells formed a layer on the HCT-8 cell surface, reducing L. monocytogenes in the surrounding environment. These findings suggest that L001 not only produces antimicrobial compounds but also limits pathogen colonisation through a physical barrier effect. Although ampicillin showed stronger antimicrobial activity, L001 effectively suppressed pathogen growth and offers a natural alternative with a lower risk of resistance. At the same time, its regular consumption may support immunity and help prevent infections. Overall, L001 shows dual potential: heat-inactivated cells that can limit pathogen colonisation through a physical barrier effect, while its metabolite extract provides broad-spectrum antimicrobial activity, together offering a natural approach to improve food safety, enhance infection control, and support immune protection.

Keywords: Foodborne diseases; lactic acid bacteria; Listeria monocytogenes

Evaluation of IgE, Mast Cells, and Allergy-Related Cytokines in Vaccinated and COVID-19 Recovered Individuals

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ABSTRACT

Despite extensive study of SARS-CoV-2 infection and vaccination, the impact on allergic immune responses and post-infection immune regulation remains unclear. In this study, we examined allergy-related immune markers in vaccinated individuals with prior COVID-19 infection. Total IgE, specific anti-SARS-CoV-2 IgE (sIgE), and pro-inflammatory cytokines (IL-17A, IL-8, IL-2, IL-4 and IL-6) were measured using ELISA, while mast cells were quantified by flow cytometry. Peripheral blood mononuclear cells (PBMCs) were stimulated with SARS-CoV-2 spike protein to assess cytokine responses. Total IgE and sIgE levels were significantly elevated in younger individuals compared to older individuals. Similarly, males showed significantly higher total IgE and sIgE than females. A history of allergy was significantly associated with increased total IgE but not with sIgE. Meanwhile, mast cell levels did not differ significantly by gender, age, allergy status, or infection severity. Upon peptide stimulation, IL-4 levels were significantly increased, especially in individuals with a history of severe infection, suggesting a Th2-skewed shift upon re-exposure, potentially impacting post-COVID-19 immune regulation and hypersensitivity. Other cytokines (IL17A, IL-8, IL-2 and IL-6) showed no significant differences, though some showed upward or downward trends. These findings suggest post-COVID-19 immune alterations that may affect hypersensitivity and other immune-mediated conditions, which, together with age and allergy-related differences in IgE levels, underscore the need for further research to guide treatment strategies and clinical management of at-risk individuals.

Keywords: COVID-19; cytokines; IgE; hypersensitivity; mast cells; SARS-CoV-2

Elucidating Anthocyanin Biosynthesis in *Sauropus* androgynus Using Systems Biology Approach

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ABSTRACT

Sauropus androgynus, a tropical medicinal plant native to Southeast Asia, holds significant potential due to its traditional use and health-promoting phytochemicals. It is particularly rich in dietary flavonoids, especially anthocyanins, which act as antioxidants and natural colorants, highlighting its value as an underutilised resource in the region. Despite its medicinal importance, there is limited understanding of the specific anthocyanin compounds present and their biosynthetic pathways. This study employed a multidisciplinary approach, combining metabolomics, microscopy, and transcriptomics to elucidate anthocyanin biosynthesis in S. androgynus. LC-MS analysis identified six specific anthocyanins, with pelargonidin-3-O-glucoside, cyanidin-3-O-glucoside, and delphinidin-3-O-glucoside as the predominant compounds. Cross-sectional microscopy revealed anthocyanin localisation primarily in the epidermal and cortex layers of plant tissues. Transcriptome sequencing via Illumina HiSeq uncovered all key genes involved in anthocyanin biosynthesis, including F3H, DFR, LDOX, and UFGT, which were selected for further in silico characterisation. Structural modeling indicated high similarity to known enzymes, with validated 3D conformations suitable for future functional studies. Furthermore, synthetic constructs incorporating these biosynthetic genes were designed into a single plasmid, each driven by unique promoters, setting the stage for in vitro overexpression of anthocyanins. This integrated approach enhances understanding of the molecular pathways in S. androgynus and offers a promising platform for producing high-purity anthocyanins, supporting its potential in nutraceutical, cosmetic, and medicinal applications.

Keywords: Anthocyanin; liquid chromatography; next generation sequencing; synthetic biology

Diagnostic Performance of Antibody-Based Rapid Tests for *Strongyloides stercoralis*: A Global Systematic Review and Meta-Analysis

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ABSTRACT

Strongyloides stercoralis infection remains a neglected tropical disease with significant morbidity, especially in endemic and resource-limited areas. Conventional diagnostic techniques are technically and resource-challenging, prompting interest in rapid diagnostic tests (RDTs) for timely detection. This systematic review and meta-analysis aimed to evaluate the current available diagnostic performance of RDTs for S. stercoralis. The review was conducted following the PRISMA 2020. A search was conducted in PubMed, Scopus, and Web of Science. Studies assessing RDTs against a reference standard were included. Eligible studies include papers that report sensitivity and specificity. The quality assessment was evaluated using QUADAS-2. Meta-analyses were performed in R using a bivariate random-effects model. Fourteen studies published between 2019 and 2025, representing diverse geographic settings, were included. Sample types were predominantly serum, with antigen targets such as recombinant NIE, Ss1a, SsIR, and S. ratti. The pooled sensitivity and specificity for all RDTs were high, with IgG-based tests showing sensitivity of ~92% and specificity of ~90%, while IgG4-based assays had slightly lower pooled sensitivity (~88%) but comparable specificity (~93%). Recombinant NIE-IgG4 assays demonstrated powerful performance in specific settings. Subgroup analyses revealed antigen type, antibody class, and population risk profile variations. RDTs offer promising accuracy for S. stercoralis detection, potentially enabling broader screening where advanced laboratory methods are unavailable. RDTs, particularly recombinant NIE-based formats, are reliable for S. stercoralis diagnosis and could strengthen control strategies in endemic areas. Further standardisation and field validation are warranted to enable widespread implementation and global control efforts.

Keywords: Antibody; neglected tropical diseases; rapid diagnostic test; recombinant NIE antigen; *Strongyloides stercoralis*

Field Validation of a New Brugia Rapid Test (BT+) for the Detection of Lymphatic Filariasis in Malaysia

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ABSTRACT

The parasitic nematodes Wuchereria bancrofti, Brugia malayi and Brugia timori cause vectorborne lymphatic filariasis (LF). A sensitive point-of-care rapid test is needed to detect infection, recrudescence and interrupt disease transmission in endemic areas. This study aimed to validate the performance of a new rapid test (BT+). A cross-sectional study was conducted in eight LF endemic areas in Beluran and Pitas, Sabah. Venous and capillary night blood samples were collected from 808 eligible participants aged 5 years and older who had resided in the study sites for a minimum of six months. Venous blood in EDTA tubes was used for rt-PCR, BT+ and Brugia rapid test (BRT). BT+ was evaluated using eluted dried blood spots (DBS) and plasma samples. Finger-prick capillary blood was obtained for microscopic examination using thick blood smear (TBS) and on-site BT+ testing. The sensitivity of BT+ was 100% in comparison to TBS and rt-PCR. The overall positivity for BT+ was 42.5% (95% CI: 39.01-45.94), and BRT, 47.6% (95% CI: 44.09-51.09). Adults exhibited higher LF positivity than children for both BT+ and BRT. In microfilaria-negative or PCR-negative samples, BT+ detected 65.5% of IgG4 antibodies whereas BRT detected 59.5%. The Kappa statistics shows substantial agreement between BT+ and BRT, k=0.783, p-value<0.001. Positive blood samples across the matrices were 96% concordant, with only one DBS sample yielding a negative result. In contrast, all negative blood samples across the matrices showed 100% concordance. The study findings provide evidence for the future diagnostic tool in the national LFEP control program in Malaysia.

Keywords: Brugia malayi; lymphatic filariasis; rapid diagnostic test

Strongyloidiasis Knowledge and Preparedness among Physicians: Early Insights from a Malaysian Tertiary Hospital

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ABSTRACT

Strongyloidiasis, caused by the nematode Strongyloides stercoralis, is a public health concern, affecting over 600 million people globally. Despite being included in the World Health Organisation's (WHO) Neglected Tropical Disease (NTD) Roadmap 2021-2030, strongyloidiasis remains underrecognised and underdiagnosed in many healthcare settings. It is particularly problematic as the disease poses a significant risk to immunocompromised patients. Consequently, adequate knowledge among physicians is crucial for timely diagnosis and effective management. This study aimed to evaluate physicians' knowledge of strongyloidiasis management and identify factors influencing their awareness and clinical preparedness in a tertiary care setting. A cross-sectional study was conducted among 70 physicians at the Hospital Canselor Tuanku Muhriz (HCTM), Kuala Lumpur, Malaysia. A validated, structured questionnaire hosted on Google Forms was used to assess socio-demographics, familiarity, and knowledge of Strongyloides infection. Knowledge scores were categorised according to Bloom's taxonomy and subsequently analysed using SPSS (version 27.0). Our findings show that 41% of physicians demonstrated adequate knowledge. Familiarity with the disease (50%), experience treating immunocompromised patients (45%), and prior training in tropical medicine (56%) were associated with higher knowledge scores, although none were statistically significant (p > 0.05). Neither clinical experience (38%) nor advanced qualifications (40%) showed a significant benefit. These preliminary findings revealed knowledge gaps among physicians regarding strongyloidiasis. Although the observed associations were not statistically significant, the results highlight the need for increased education and awareness. Future large-scale research is essential to fully assess these knowledge gaps and inform training needs.

Keywords: Immunocompromised; knowledge assessment; physicians; *Strongyloides stercoralis;* strongyloidiasis

Recurrent Lymphatic Filariasis in the Implementation Unit (IU) Tangkarason, Sabah

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ABSTRACT

Lymphatic filariasis (LF) remains a significant health concern in tropical and subtropical regions, caused by the mosquito-borne nematodes, Wuchereria bancrofti, Brugia malayi and Brugia timori. Malaysia has made significant progress towards LF elimination via the implementation of mass drug administration (MDA). Persistent infection pockets persist in certain areas, such as IU Tangkarason, Sabah. We reviewed and analysed retrospective data from the LF surveillance and research reports on LF elimination at IU Tangkarason, spanning the years 2003 to 2024. A total of 33 villages in IU Tangkarason had undergone the Lymphatic Filariasis Elimination Programme, encompassing a population of 6,404 residents. The MDA coverage from 2003 to 2016 ranged from 97% to 99%. A mini-transmission assessment survey in 2018 indicated an 8% LF positivity rate. The MDA with triple-drug therapy was subsequently introduced in 2018, achieving coverage rates of between 92% and 100%. Post-MDA surveillance utilising the triple-drug therapy continues to reveal LF prevalence exceeding 2%, with positivity rates ranging from 0.6% to 28.6% in 2019, 0.8% to 22% in 2021, and 1.7% to 20% in 2022. In 2016, animal blood sampling and microscopy indicated LF positivity in cats (6.7%) and dogs (54%), while the overall animal positivity rate for B. malayi DNA by PCR 9.4%. The persistent LF in the IU Tangkarason indicates continuous active LF transmission. Effective strategies are essential, including incorporating "One Health" initiatives to ensure the successful elimination of LF in endemic areas.

Keywords: Elimination programme; lymphatic filariasis; surveillance; Tangkarason Sabah; zoonoses

Disseminated Strongyloidiasis in Immunocompromised Patients: A Case Series from Malaysia

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ABSTRACT

Strongyloides stercoralis infection is usually asymptomatic or presents with mild gastrointestinal symptoms in immunocompetent hosts. However, in immunocompromised individuals, dissemination can occur, leading to severe and often fatal outcomes. Disseminated strongyloidiasis remains underrecognised in Malaysia due to diagnostic challenges and overlapping clinical manifestations with other infections. We describe a case series of disseminated strongyloidiasis in Malaysian patients with underlying immunocompromised states. The patients presented with nonspecific features, including abdominal pain, diarrhoea, fever, and respiratory distress. Risk factors included prolonged corticosteroid therapy, haematological malignancy, and advanced chronic illnesses. They were initially misdiagnosed as bacterial sepsis or pulmonary tuberculosis. Laboratory confirmation was achieved through the detection of larvae in stool, sputum, and bronchoalveolar lavage specimens. Despite initiation of anti-helminthic therapy with ivermectin and albendazole, all three patients died due to overwhelming infection. These cases illustrate the high morbidity and mortality associated with disseminated strongyloidiasis in immunocompromised patients. Early diagnosis is difficult, particularly in settings where clinical suspicion is low. Routine screening for Strongyloides infection prior to initiating immunosuppressive therapy and heightened awareness among clinicians and laboratory personnel are essential in preventing fatal outcomes. Disseminated strongyloidiasis represents a serious and potentially preventable infection in immunocompromised Malaysian patients. Vigilance, early recognition, and timely treatment are key to improving prognosis in this vulnerable population.

Keywords: Disseminated strongyloidiasis; immunocompromised patients; Malaysia; *Strongyloides* stercoralis

Axillary Cutaneous Larva Migrans

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ABSTRACT

Cutaneous larva migrans (CLM) is a common dermatoses in tropical regions, typically affecting the distal lower extremities. It is caused by the accidental dermal penetration and migration of canine hookworm larvae, primarily *Ancylostoma braziliense* and *Ancylostoma caninum*. We report a case of CLM with an uncommon axillary presentation in a 61-year-old durian farmer from a rural area. The patient presented with pruritic, erythematous serpiginous lesions over the right axilla, persisting for over two weeks. Initially misdiagnosed as a fungal infection, his symptoms worsened with antifungal treatment before seeking a second opinion. A detailed history revealed significant soil exposure during durian tree planting. The diagnosis of CLM was made clinically, and treatment with oral albendazole and antihistamines resulted in marked symptom resolution within one week. This case underscores the diagnostic challenges posed by atypical presentations of CLM and highlights the importance of thorough clinical history and awareness of zoonotic dermatoses. Physicians, especially in tropical and rural settings, should consider CLM in patients with serpiginous skin lesions even at uncommon anatomical sites such as the axilla to avoid misdiagnosis and ensure prompt treatment.

Keywords: Axillary; CLM; dermatoses; larva migrans; zoonotic

Diagnostic Challenges in Non-Endemic Helminthiases: A Case Series from Malaysia

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ABSTRACT

Helminthiases are predominantly associated with endemic regions; however, increased migration, globalisation, and international travel have led to the emergence of unusual parasitic infections in non-endemic countries. In Malaysia, such cases are rare and pose significant diagnostic challenges due to their unfamiliarity and atypical clinical presentation. We report three cases of non-endemic helminthiases encountered in Malaysia. The first involved a pulmonary hydatid cyst caused by Echinococcus granulosus, initially misdiagnosed as a malignant lung lesion. The second case described the incidental detection of Diphyllobothrium latum eggs in the urine of a young patient who presented with nonspecific urinary symptoms. The third case involved the misidentification of Schistosoma japonicum ova in stool as hookworm ova. Each case demonstrated unique diagnostic pitfalls, particularly in differentiating from common infectious or neoplastic conditions. These cases highlight the importance of maintaining a broad differential diagnosis when evaluating atypical clinical presentations in a non-endemic setting. The rarity of such infections may result in delayed or missed diagnoses if clinicians and laboratory personnel lack awareness of their parasitological features. Detailed travel history, familiarity with uncommon helminth morphology, and close collaboration between clinicians and parasitologists are essential for accurate diagnosis and timely management. Non-endemic helminthiases, though uncommon in Malaysia, remain clinically relevant. Greater awareness and diagnostic vigilance are necessary to prevent misdiagnosis and ensure optimal patient care.

Keywords: Case series; diagnostic challenges; helminthiases; Malaysia; non-endemic

In Silico Identification and Plasmid Construction of Nipah Virus G Protein Epitope

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ABSTRACT

Nipah virus (NiV), a zoonotic paramyxovirus, causes recurrent outbreaks in South Asia with 40-100% case fatality rates, posing a significant pandemic risk. While mRNA, protein subunit, and viral vector vaccines are in early clinical trials, no epitope-based candidates have reached human testing. This study reports the design and construction of a linear B-cell epitope vaccine targeting the NiV G glycoprotein, a key neutralising antigen. BepiPred 2.0, ABCpred, and SVMTrip identified the core B-cell epitope "DAFLIDRINW" from the NiV G protein. Molecular docking using AutoDock Vina assessed its interaction with the B-cell receptor (BCR). Solubility check using NetSolP and exclusion of disulfide bonds guided the construct design. MHC-II binding was predicted using the IEDB Consensus method. To facilitate cloning and expression, flanking residues were added, resulting in the synthetic construct sequence ("WEGVYNDAFLIDRINWISAG"). This construct was designated as GE1. The GE1 sequence was subsequently cloned into pET28a to produce the recombinant plasmid pET28a-GE1, expressed in Escherichia coli RosettaBlue (DE3). The core epitope demonstrated high prediction scores and formed a stable BCR complex (-7.8 kcal/mol). GE1 showed favorable solubility (0.54), no disulfide bonds, and strong predicted binding to multiple HLA-DR alleles. The pET28a-GE1 plasmid was successfully generated, with sequencing confirming correct in-frame insertion. The identified GE1 construct demonstrates strong theoretical potential as a peptide-based subunit vaccine. This bioinformatics-guided workflow demonstrates how computational pipelines can accelerate early-stage vaccine discovery, enabling rapid responses against high-fatality pathogens such as NiV.

Keywords: Bioinformatics; humoral immune response; linear B-cell epitopes; molecular docking; Nipah virus glycoprotein

Pan Sarbecovirus Epitope Discovery Using Reverse Vaccinology Approach

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ABSTRACT

Over two decades, coronaviruses have caused three serious outbreaks, namely severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Pan-sarbecoviruses, including SARS-CoV-2, pose an ongoing threat to global health due to their high potential for cross-species transmission and pandemic emergence. Its Spike (S) protein is known for constant mutation, especially variants of concern (VOCs), which becomes a significant challenge to the development of a vaccine as it exhibits increased transmissibility and virulence and often escapes antibody protection from previous infection or vaccination. The development of broad-spectrum vaccines requires the identification of conserved neutralising epitopes capable of eliciting cross-protective immunity. In this study, the reverse vaccinology approach was used to identify candidate epitopes from known antibody-binding regions across representative Sarbecovirus spike protein sequences. The antibodybinding sites from the literature and structural databases were mapped onto multiple Sarbecovirus and MERS-CoV spike protein sequences retrieved from the NCBI GenBank. Using multiple bioinformatic tools, the conserved regions were aligned and screened for B-cell, MHC I, and MHC II epitopes prediction. Epitopes which were predicted to be antigenic, non-allergenic, and non-toxic were selected for vaccine design. We demonstrated the utility of reverse vaccinology in rapidly identifying conserved, potential protective epitopes from antibody-binding regions, supporting the design of pan-sarbecovirus vaccines. The methodology and findings from this study may inform future computational and experimental efforts targeting other high-risk viral families with pandemic potential.

Keywords: Bioinformatic; epitope; SARS-CoV; SARS-Cov-2; vaccine design