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AGENT BASED MODELLING: AN *IN SILICO* STUDY ON THE EFFECTIVENESS OF *WOLBACHIA*-INFECTED MOSQUITOES AS VECTOR CONTROL AGENTS

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Background:
Dengue Fever (DF) is a mosquito-borne disease which is a major epidemic concern especially in the South East Asia region. Various control intervention have been implied to control the breeding of mosquito vectors including the application of *Wolbachia*-infected mosquitoes to reduce the transmission of dengue fever in Malaysia. Using the available data on the life cycle and transmission pattern of *Aedes* mosquitoes, this study explore the probability of the interaction of releasing *Wolbachia*-infected mosquitoes with dengue-infected mosquitoes spatially, and how would this interaction be able to reduce the case of dengue fever.

Methods:
A simple model is designed based on epidemiological data available and introduced in agentbased modelling. The model operates at local scale with support of computational infected mosquito with *Wolbachia* and with dengue virus. All the agents presented in the framework are in a scale of 1000 per agent, supported by GAMA Modelling and simulation platform (http://gama-platform.org), scheduled using a time scale of 60 minutes over 6 months period.

Results:
The model predicted that 96% of dengue-infected mosquito will be replaced by *Wolbachia*infected mosquito over a period of 6 months. In this study, the usage of GAMA as a new approach for disease modelling was explored. The steps and methods used to create a simulation to predict the effectiveness of introducing *Wolbachia* to wild mosquitoes in a certain area in order to reduce the incidences of dengue fever were described. To some degree, computer simulations such as GAMA can be seen as an experimental procedure for hypothesis testing and scenario analysis, as well as for the understanding of a complex system, which can be utilized in combination with experiments in real-life.

Conclusion:
The result proposed that the release of *Wolbachia*-infected mosquito into dengue hotspot population may reduce the case of dengue fever. This model has been designed to be completely generic and applicable to any geographic setting at any spatial scale. More modification of parameters will be implemented and the length of the exposure *in silico* will be expanded for five to ten years period to increase the strength of the model.

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ISOLATION AND IDENTIFICATION OF BACTERIAL CONTAMINATION FOUND ON THE SURFACE OF MALAYSIAN PAPER CURRENCY COLLECTED FROM LOCAL NIGHT MARKETS IN SHAH ALAM, SELANGOR

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Background:
According to Bank Negara (2016), paper currency is used globally as an exchange for goods and services in their respective countries. Thus, paper currency is suggested to be a potential vehicle for microbial transmission. This study provides a better view on the potential of Malaysian ringgit to harbor possible pathogenic bacteria.

Methods
This cross-sectional study focused on studying the morphological and biochemical identification of microbial present on Malaysian ringgit. Sixty samples of five denominations (RM 1, RM 5, RM 10, RM 20 and RM 50) were randomly collected from 3 night markets in Shah Alam area. The samples collected were separated according to three subcategories; meat, seafood and vegetable vendors. Microbiological procedure was adapted based on Tamalli et al. (2012) and samples were inoculated into Nutrient agar (NA), Mannitol Salt agar (MSA) and MacConkey agar, then subjected to Gram staining and further biochemical tests.

Results:
Out of 60 samples inoculated, 55 samples (91.6%) were found to consist of various pathogenic bacteria. Out of these 55 notes, 44 samples (80%) were polymicrobial and 11 samples (20%) was unimicrobial. Some examples of the bacterial isolates include *Escherichia coli, Klebsiella* sp., *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella* sp., *and Streptococcus* sp. with *S. aureus* being the most common isolated bacteria (19%). Bacterial contamination frequency was observed to be higher in smaller paper denominations (RM 1) due to the increased frequency of currency exchange between vendors and consumers as compared to other notes studied.

Conclusion:
The isolation of bacteria in these paper notes confirmed various studies that paper currency can play a vital role in bacterial transmission of potential pathogenic bacteria. This study also revealed that there is no association of the types of vendors with the bacterial isolates found.

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PRELIMINARY STUDY OF NRAMP1 GENE POLYMORPHISMS WITH SUSCEPTIBILITY TO TUBERCULOSIS IN HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM) POPULATION

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Background:
Tuberculosis (TB) disease is a major health problem caused by the human pathogen *Mycobacterium tuberculosis* (Mtb) infection. It is transmitted primarily via the respiratory route that can attack pulmonary and extra pulmonary sites. Many genetic studies had demonstrated that Natural ResistanceAssociated Macrophage Protein 1 (NRAMP1) gene polymorphisms as one of the risk factors for TB development. Therefore, this study aims to investigate the association of NRAMP1 gene polymorphisms with TB susceptibility in HUSM population.

Methods:
Whole blood samples were collected from 16 confirmed TB patients, 15 purified protein derivative (PPD) negative healthy contact group as control group and 15 PPD positive healthy contact group from HUSM. Genomic DNA was isolated from the blood samples and PCR was performed prior to pyrosequencing via Pyromark Q96ID. In this preliminary study, three SNPs sites of NRAMP1 gene, NRAMP1-3, NRAMP1-4 and NRAMP1-5 were investigated and analysed among the three groups. The SNPs sites were detected in the form of signal peak in Pyrogram trace and mutated nucleotide sequence.

Results:
The SNPs allele and genotype frequencies of NRAMP1 gene among the three groups were compared analysed using Pearson's Chi-Square test. As the result of the findings, the three SNPs sites, NRAMP13, NRAMP1-4 and NRAMP1-5 did not showed any significant association with TB susceptibility among the three groups observed.

Conclusion:
This preliminary study suggested that the three SNPs sites did not show any significant association with TB susceptibility. Some differences were also detected between studies by various researchers, showing mutation in other genes, probably due to ethnic differences in different geographical areas. Therefore, a large sample size of TB patient and healthy contact are necessary to confirm the association of NRAMP1 polymorphisms.

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THE EFFECT OF *Centella asiatica* ON Bacille Calmette Geurin (BCG) AND RECOMBINANT BCG EXPRESSING THE MSP-1C OF *Plasmodium falciparum*

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Background:
An attenuated of *Mycobacterium bovis* bacille Calmette-Guerin (BCG) is the only vaccine used for tuberculosis. It represents one of the most promising live vectors for the delivery of foreign antigen to the immune system, including malaria parasites. Previously, our group has constructed a recombinant BCG (rBCG) clone that expresses the MSP-1C of *Plasmodium falciparum*. This study was conducted to determine the effect of *Centella asiatica* (*C. asiatica*) extract, a medicinal plant that traditionally used for the treatment of variety disorder including infections, inflammation and immune system deficiencies on BCG and rBCG cultures.

Methods:
The direct effect was determined by evaluating the minimum inhibitory concentration (MIC) using MTT assay and minimum bactericidal concentration (MBC) using colony forming unit (CFU), while the indirect effect was determine on protein profiling of the mycobacterium using SDS-PAGE.

Results:
The results of this study showed that the *C. asiatica* extract reduced the growth of the BCG and rBCG cultures in a concentration-dependent manner. However, the highest concentration of the plant extract does not inhibit the growth of the BCG and rBCG colonies on 7H11 media. In addition, the highest and the lowest concentration of *C. asiatica* extract also do not change the protein profile of the BCG and rBCG. These data demonstrate that *C. asiatica* extract does not significantly affect the growth of the BCG and rBCG cultures.

Conclusion:
Therefore, this plant extract is safe to be consumed as “ulam” or dietary supplements because it might not interfere with the vaccines in our body.

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BACTERIAL AND FUNGAL CONTAMINATIONS OF CELL CULTURES IN SCHOOL OF HEALTH SCIENCES, USM.

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Background:
Biological cell culture contamination is a major issue in cell culture studies. The objective of this study was to determine the percentage of cell culture contamination by bacteria and fungi in cell culture laboratory of School of Health Sciences, USM.

Methods:
A total of 32 contaminated cell cultures of different cell lines and passages were selected based on macroscopic observation and collected over 6 months duration. Microbial culture was performed directly from cell culture media and also cell pellets after centrifugation by inoculating onto the blood agar, MacConkey agar and Sabouraud dextrose agar. The identification of each isolated bacterium or fungus was conducted using conventional microbiological techniques.

Results:
This study revealed 84.4% of the samples were positive for bacterial and/or fungal contaminations. Macroscopic observations showed changes in colour, pH and cloudiness of the culture media. The bacterial, fungal and mixed contaminations were 35.5%, 34.4% and 12.5% respectively. The contaminating bacterial agents were *Bacillus* sp., *Moraxella* sp., *Pseudomonas* sp. *Acinetobacter* sp. and *Serratia* sp. The fungal contaminants isolated were *Aspergillus* sp., *Penicillium* sp. and *Candida* sp. Cell culture of lower passage and shorter culture day were most frequently contaminated. RPMI media were found to have highest contamination compared to other culture media. The most commonly affected cell lines were MDA-MB231 and SiHa.

Conclusions:
This is the first report of cell culture contamination from our cell culture laboratory. A high percentage of contaminations indicates the need for more extensive study to identify the sources and measures to control its frequent occurrences in future.

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LARVICIDAL STUDY OF *MURRAYA KOENIGII* ETHANOLIC EXTRACT ON WILD STRAIN MOSQUITOES AROUND KUANTAN

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Background:
Vector borne diseases like dengue, malaria and chikungunya had widely spread to the entire world. Many solutions had been introduces to eliminate all the vectors but it still cannot make it reduce in number. One of the methods that had been used to control spread of the diseases is by targeting larval stages of mosquitoes. Abate is one classic example of larvicide widely used in eradicating mosquito larvae. So, the main objective of this study is to determine the larvicidal activity of ethanolic extract of curry leaves (*Murraya koenigii*) against wild strain mosquitoes around Kuantan as one of alternative approaches.

Method:
M. koenigii leaves were subjected to 3 days maceration using absolute ethanol. The macerated *M. koenigii* then filtered and evaporated using rotary evaporator to produce crude extract. The crude extract undergo few series of dilutions with absolute ethanol as solvent to produce test concentrations (100 ppm, 50 ppm and 10 ppm). Absolute ethanol and abate served negative and positive control respectively for bioassay. The mosquitoes larvae were collected around Kuantan that was the habitat of mosquitoes and potential habitat for them.

Results:
The ethanolic extract of curry leaves (*Murraya Koenigii*) solution was induce 100% mortality to larvae in just 24 hours.

Conclusion:
There was a significant larvicidal activity of ethanolic extract of *M. koenigii* on wild strain mosquitoes.

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Isolation of Bacterial Contaminants from Elevator Buttons of Kuliyyah of Medicine and Office of Campus Director in International Islamic University Malaysia Kuantan

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Background:
There had been an increase in the awareness of microbial contamination in the world today. It can often go unnoticed or be found in the most unexpected places. With the increase of infectious disease globally, it is more important for us to take preventive measures in order to prevent further spreading of bacterial contamination. In this study, we have identified and measured the bacterial contamination that can be found on the surface of elevator buttons of Kuliyyah of Medicine and Office of Campus Director in International Islamic University Malaysia Kuantan Campus (IIUMK).

Methods:
The study was a cross sectional research. Samples were taken from elevator buttons from surface of elevator buttons of Kuliyyah of Medicine and Office of Campus Director in IIUMK using sterile cotton swab moisten with nutrient broth. Enumeration of bacteria was done using viable plate count method. The enumeration of bacterial contaminants was recorded as CFU/ml and classified into low, medium or high contamination level. Morphology characteristic of bacterial contaminants was observed by streaking bacterial colonies on mannitol salt agar and MacConkey agar according to the standard microbiological methods. Finally, Gram staining and PCR amplification of 16rRNA were carried out to further distinguished and identified the type of bacteria.

Results:
This study was conducted on 89 buttons from five elevators available in Kuliyyah of Medicine and Office of Campus Director in IIUMK. Eighty four percent of the samples showed growth of bacterial contamination. Enumeration of bacteria was categorised into low, medium and high contamination categories. Fifty elevator buttons were categorised having low contamination and twelve elevator buttons are classified as having medium contamination. Twelve elevator buttons is categorised into high contamination category. It is expected that the elevator buttons were dominated by Staphylococcus and Enterobacter species based on morphological characteristics and Gram types. Molecular identification via PCR is still underway.

Conclusions:
Preliminary result revealed there is a presence of bacterial contaminants on the surface of elevator buttons of Kuliyyah of Medicine and Office of Campus Director in IIUMK. Hence, elevator buttons could be the transmitting medium of pathogenic bacteria. The findings signify the importance of routine elevator buttons disinfection to lessen bacterial transmission. Keywords: microbial contamination, elevator, university

Isolation of Bacterial Contaminants from Elevator Buttons of Faculty of Dentistry and Faculty of Allied Health Sciences in IIUM Kuantan

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Background:
Large buildings such as hospitals, universities, malls and health care centers tend to have elevators as the main transportation to connect people to places with substantial traffics almost every day. Elevator button surfaces in medical, educational and community settings may be regarded as under-recognized sites of bacterial contamination. However, recent studies have proven otherwise whereby high prevalence of bacterial colonization is observed. Frequently used public surfaces are the most common bacterial sources for infection transmission through direct or indirect contact with clothes and exposed skin such as fingertips. Through this study, isolated bacterial contaminants from the elevator button surfaces of dentistry and allied health sciences faculties were investigated, implementing awareness of importance of hand hygiene as well as disinfecting management of the elevator button surfaces in the community.

Methods:
The sampling procedure was derived from Mustafa et al., (2015). The sampling was done in duplicate from the surface of elevator buttons of dentistry and allied health sciences faculties. Bacterial enumeration of each elevator button was performed using plate count method. Colonies from plate counting procedure were streaked on MacConkey and mannitol salt agars, followed by Gram staining for preliminary identification based on morphology characteristics and Gram types.

Results:
Average number of bacterial colonies on nutrient agar plate ranged from zero to 80 CFU/ml. The numbers of elevator buttons having low, intermediate or high contamination level were 15, 51 and 32 respectively out of 98 plates. Forty-eight isolates were identified as Gram-positive *Staphylococcus aureus*, followed by sixty-two isolates of coagulase-negative Staphylococcus species and thirty isolates of Gram-negative bacteria.

Conclusion:
The findings showed the present of potential harmful bacteria on the surface of elevator buttons of two faculties in IIUM. Elevator buttons could be the medium of bacterial transmission from a person to another. Hence, through this study, the importance of hand hygiene and routine disinfection could be highlighted even more.

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The Production of Biofilm in Relation to Serotype of *Streptococcus Pneumoniae*: A Preliminary Study

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Background:
Streptococcus pneumoniae is one of the problems in healthcare sector which can lead to the acute otitis media, pneumonia, sepsis, and meningitis. *Streptococcus pneumoniae* is a Gram positive bacteria which commonly colonize human respiratory tract and has ability to form biofilm. This pneumococcal biofilm become a great concern because it was difficult to be eradicated due to antimicrobial and antibiotic resistance. Pneumococcal biofilm refers to the aggregation process of *S. pneumoniae* which attached to a surface and composed of extracellular polymeric substance (EPS), polysaccharide and nucleic acid. The previous study showed that the production of biofilm will be varying based on their serotype. This study aims to determine the association of pneumococcal biofilm with their serotype.

Methods:
This preliminary study involved 12 strains of *S. pneumoniae* which come from different serotype. The production of pneumococcal biofilm was determined by measuring optical density using microtiter plate method. From the value of optical density, biofilm production was categorized into four categories of biofilm namely non-adherent, weak, moderate and strong biofilm producers. The association between pneumococcal biofilm and their serotype was determined.

Results:
The preliminary result showed, 3 (25%) strains as non-adherent, 3 (25%) strains as weak and 6 (50%) as moderate biofilm producer. None of the strains produced strong biofilm formation. Among the moderate biofilm producer, 19A serotype exhibit more biofilm.

Conclusion:
Different serotype of *S. pneumoniae* may contribute to the different level of pneumococcal biofilm formation.

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Comparison Analysis of Bacterial Hyaluronidase between Staphylococcus aureus and Staphylococcus epidermidis Isolated from Healthy Adult

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Background:
Hyaluronidase is an enzyme that is produced by living organism including human, animals and also bacteria. It plays a role in catalyzing the breakdown of hyaluronic. At some point, hyaluronidase could affect the health of human by facilitate the process of infection. However, with advancement of scientific studies and researches, the mechanism of hyaluronidase had been exploited and be implemented in health care to treat various diseases and other health conditions. In this aspect, the scientists and researchers had discovered the commercial values of this enzyme and use it in therapeutic uses for instance in deliver the drugs to the targeted sites. However, there are some concerns regarding the uses of commercial hyaluronidase that available at this present time. This is because most of them which are produced from animal sources had showed some side effects from the usage and at the same time, it raises the halal issues for the Muslims regarding the sources or the production process. Therefore, this study was conducted in order to investigate the potential value of bacteria to be used as sources of hyaluronidase by screening and comparing hyaluronidase production in commensal *Staphylococcus aureus* and *Staphylococcus epidermidis* from skin and respiratory related-niches of healthy male and female adults.

Methods:
A total of 108 swab samples were collected from anterior nares and palm and identified. The isolates were screened for hyaluronidase production using hyaluronic acid diffusion rapid plate method.

Results:
Based on the result, high isolation prevalence of commensal *S. aureus* and *S. epidermidis* were observed among healthy adult with equal prevalence number in different niche of isolation and gender of the host. High number of isolates produced hyaluronidase regardless of niche and host gender whereby it showed that approximately 63% of *S. aureus* and 54% from *S. epidermidis* give positive result of hyaluronidase production.

Conclusion:
It can be concluded that commensal *S. aureus* and *S. epidermidis* revealed their abilities as hyaluronidase producer without a significant involvement of bacterial niche and gender of the host.

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ASSESSING THE ANTIBACTERIAL ACTIVITIES OF ZINC OXIDE, TUNGSTEN OXIDE AND ZINC OXIDE-TUNGSTEN OXIDE NANOPARTICLES TOWARDS *STAPHYLOCOCCUS AUREUS*

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Background:
Clean water has become scarce in developing countries such as Malaysia. Industrial and agricultural waste have severely contaminated the fresh water sources in the nation. Wastewater containing organic molecules has promoted vast microbial growth. Nanomaterials such as Zinc Oxide (ZnO) and Tungsten Oxide (WOx) has attracted research interest due to their antimicrobial activity in waste water microbial inhibition. In this study, antibacterial activities and their underlying mechanisms of ZnO and WOx nanoparticles (NPs) as well as ZnO-WOx nanohybrids (NHs) were investigated, *Staphylococcus aureus* was used as the model microorganism in the study due to its abundance in polluted water.

Methods:
ZnO and WOx NPs were synthesised using the hydrothermal method. Additional, three sets of ZnO-WOx NHs were synthesised by using homogenous precipitation method with different stirring time (24, 48 and 72 hrs). The particles were characterised to identify the structural geometries. Bacterial identification was carried out for *Staphylococcus Aureus* (ATCC 29213) to confirm the strain before carrying out the antibacterial assay. By using 2-fold broth microdilution assay, concentrations of NPs and NHs (ranging from 8 µg/ml to 1024 µg/ml) were used to identify the minimum inhibitory concentration on *S. aureus*. To determine the involvement of reactive oxygen species (ROS), the microdilution assay was performed under two different conditions (i.e. without UV and UV-60 mins).

Results:
ZnO NPs and WOx NPs showed inhibition towards *S. aureus* at 128 µg/ml and 256 µg/ml respectively. As for the 3 different types of NHs, in which WOx NPs were coated with ZnO NPs, showed similar inhibitory effect at >512 µg/ml. There was no difference observed in the antibacterial activity of these NPs and NHs against *S. aureus* either with UV or without UV irradiation, suggesting ROS did not play a role in the antibacterial activity observed.

Conclusion:
ZnO NPs exhibit greater cytotoxicity than WOx NPs and ZnO-WOx NHs towards *S. aureus* possibly through the released of Zn^{2+} ions and interaction with the bacterial cell walls, resulting in potent antibacterial activity. Doping of WOx NPs with ZnO at the outer layer did not increased the potency of the antibacterial activity towards *S. aureus*. Thus, ZnO NPs represent an economical alternative, particularly in waste water treatment. Metal oxide nanoparticles, especially ZnO NPs, will play a key role in ensuring sufficient and better quality water to meet the everincreasing demand for portable water.

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THE EFFECT OF *Clarias batrachus* MUCUS AGAINST GRAM-POSITIVE BACTERIA

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Background:
Bacterial genetic content can be mutated as it will try to adapt to its surrounding environment and causes the bacteria becoming resistant towards antibiotics thus, a new source of antibacterial agents from natural sources is needed as an alternative method. Fish able to bare the aquatic environment that is full of microorganisms, yet they can still live without getting infection easily. Their main protective system in preventing infection by pathogens is by having the epidermal layer covered with slimy mucus that plays a very important role and helps to trap any microbes that attach to it. The aim of this study is to determine the effect of mucus isolated from the body surface of *Clarias batrachus*, also known as catfish against gram-positive bacteria. The previous study has shown that mucus from many species of fish shows an antibacterial effect against microorganisms such as bacteria, parasites, and fungi.

Methods:
Mucus from the body surface of *C. batrachus* is obtained and sensitivity test is done against gram-positive bacteria by using turbidity method. The optical density of the sample is determined, and growth curve of the bacteria is plotted to know either the fish mucus effect on the bacteria is bacteriostatic or bactericidal. Microdilution test is also done to determine the minimum inhibitory concentration of the fish mucus against gram-positive bacteria. Observation under scanning electron microscope (SEM) is done to know the effect of fish mucus on the bacterial surface.

Results:
The growth curve shows that the fish mucus has bacteriostatic effect against the gram-positive bacteria. Observation under SEM shows that the mucus affects the bacterial surface, and formation of the blebs can be seen.

Conclusion:
This study shows that fish mucus plays a very important role as immune system and has an effect against gram-positive bacteria. Further study needs to be done to isolate the active compound or the antimicrobial peptide (AMP) in the fish mucus so that a new antibacterial agent can be created as a new source of antibiotic that can be used clinically.

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EVALUATION OF ZINC AND SABOURAUD DEXTROSE BROTH TO INCREASE FUNGAL GROWTH IN BLOOD SAMPLE

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Background:
Bloodstream infections caused by the fungi *Aspergillus fumigatus* and *Candida albicans* are difficult to detect by blood culture due to the zinc-chelating effects of calprotectin in blood. Zinc is an essential trace element required for fungal growth. Therefore, this study aimed to determine the optimum concentration of zinc and liquid medium to increase fungal growth in whole blood sample.

Methods:
Zinc sulphate solution (0.5 µg/ml and 0.6 µg/ml $ZnSO_4$) and Sabouraud dextrose broth (SDB) were added to whole blood samples spiked with 1.0 x 10^4 *A. fumigatus* and *C. albicans* germ tubes and incubated at 37ºC for up to 48 hours. 10 µL of the blood samples were inoculated onto Sabouraud dextrose agar plates and colony forming units (CFU) counted.

Results:
SDB with 0.6 µg/ml $ZnSO_4$ increased *A. fumigatus* growth in blood sample with the highest CFU count (2.4 x 10^3 CFU/ml) observed, followed by SDB with 0.5 µg/ml $ZnSO_4$ (1.9 x 10^3 CFU/ml), as compared to SDB or blood alone (1.7 x 10^3 CFU/ml). Whereas for *C. albicans*, SDB with 0.5 µg/ml of $ZnSO_4$ had the highest CFU count (2.71 x 10^4 CFU/ml), followed by SDB alone (1.83 x 10^4 CFU/ ml), SDB with 0.6 µg/ml $ZnSO_4$ (1.71 x 10^4 CFU/ml) and blood alone (9.7 x 10^3 CFU/ml).

Conclusion:
The addition of SDB with 0.6 µg/ml and 0.5 µg/ml of $ZnSO_4$ into whole blood sample was able to increase *A. fumigatus* and *C. albicans* growth respectively. Therefore, our study demonstrates a potential fungal growth enhancing medium for culture of bloodstream fungus.

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In-vitro Replication Kinetics of Getah virus (GETV) strains in Malaysia

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Background:
Getah virus (GETV) is a mosquito-borne virus that emerged as an important equine pathogen causing recurrent outbreaks among the economically important racehorses in Japan and India. GETV infection in horses caused fever, anorexia and hind limb edema. Other than horses, GETV infection caused fetal death and reproductive failure in swine. GETV was first isolated in Malaysia in 1955. Nevertheless, there has been no report of outbreak of the GETV infection. This could be due to the under-reporting or misdiagnosis especially when the presence of GETV in Malaysia has not been thoroughly studied. Recently, we detected GETV in various species of mosquitoes captured in the forest of different regions in Peninsular Malaysia. Our preliminary findings showed that the current circulating GETV strain (B254 strain) was molecularly distinct from the old virus strain isolated in 1955 (prototype strain, M2021). In this study, we intended to investigate the *in-vitro* replication kinetic of these different GETV strains.

Methods:
The isolated GETV strains were propagated in C6/36 mosquito cell line. The viruses were harvested and the virus titer was determined using plaque assay. Replication kinetics of GETV strains were investigated in C6/36 cell line and Vero cell line by infecting the cells with GETV strains at multiplicity of infection (MOI) = 0.1. The infected cell supernatants were harvested at different time points (0, 8, 24, 48, 72 and 96 hours), and virus titer was assessed by plaque assay and qRT-PCR. The GETV growth curves over time were plotted and compared using two-way ANOVA analysis.

Results:
GETV M2021 and B254 strains formed distinct plaques with different plaque morphology; B254 strain formed relatively smaller plaques than did M2021 strain. In C6/36 cells, there was a significant difference in the interaction between the virus strains and the sampling hour [$F(5,10) = 1966.85$, $p < 0.05$]. The mean maximum virus titer of GETV M2021 and B254 was 2.64×10^7 RNA copies/µl and 3.48×10^6 RNA copies/µl, respectively. Linear regression analysis revealed that the M2021 replicated at $2.69\times10^5 \pm 1.5\times10^4$ RNA copies/µl/day while the B254 replicated at $3.87\times10^4 \pm 2.6\times10^3$ RNA copies/µl/day in C6/36 cells. In Vero cells, similarly, there was significant difference in the interaction between the viruses and the sampling hour [$F(5,10) = 110.04$, $p < 0.05$]. The mean maximum virus titer of M2021 and B254 was 4.65×10^7 RNA copies/µl and 1.45×10^7 RNA copies /µl, respectively. The M2021 replicated at $4.32\times10^5 \pm 1.1\times10^5$ RNA copies/µl/day while the B254 replicated at $1.78\times10^5 \pm 1.9\times10^4$ RNA copies/µl/day in Vero cells.

Conclusion:
Our findings indicate that GETV B254 may have different transmission potential in comparison to the GETV M2021.

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IN VITRO ANTIVIRAL ACTIVITY OF MUSHROOM EXTRACTS AGAINST COXSACKIEVIRUS A16

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BACKGROUND

Coxsackievirus A16 (CAV-16) is one of the major causative agents of Hand, Foot and Mouth disease (HFMD). The constant outbreak of the disease that occurs periodically around the world is significant public health concern. Currently, there are no licensed vaccine or specific antiviral therapy available for CAV-16. Thus, this study aims to investigate the potential antiviral activity of mushroom *G.neojaponicum* and *A.cinnamomea* extracts against CAV-16 and to determine the antiviral mechanism of the selected extracts against CAV-16.

METHODS

The cytotoxic effect of *G.neo-japonicum* and *A.cinnamomea* against Vero cells was tested at various concentrations to determine the maximum non-toxic dose that allowed 100% of viability. The screening of antiviral activity of the selected mushroom extracts at non-toxic dose was screened by observing the reduction of viral cytopathic effect (CPE). Mushroom extracts with antiviral properties were then characterized by pre-treatment, virucidal and post-treatment assays to determine the antiviral mechanism. The extracts that caused reduction in CPE were further quantitated by viral titration (cell culture infectious dose 50%).

RESULTS

Both *G.neo-japonicum* and *A.cinnamomea* extracts depicted a reduction in CPE at 1.25 mg/ml of *G.neo-japonicum* and 2.5 mg/ml of *A.cinnammomea*. However, the *G.neo-japonicum* extract was selected for characterization of its antiviral properties as it is a local variety of mushroom and appears to be more potent antiviral effects compared to *A.cinnamomea* . An obvious reduction in CPE was observed in post-treatment assay. The result was supported by viral titration which showed lower viral titre in treated cells ($7.08X10^5$ $CCID_{50}$/ml) than in untreated control ($2.11X10^6$ $CCID_{50}$/ml). No obvious reduction in CPE was observed in pre-treatment and virucidal assays.

CONCLUSION

The mushroom, *G.neo-japonicum* ethanolic extract shows promising antiviral effects against CAV-16 in post-treatment assay suggesting that the antiviral effect is due to inhibition of CAV-16 replication cycle. This mushroom extract may serve as a potential therapeutic agent against CAV-16. However, further investigations are needed to confirm this.

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IN VITRO STUDY OF ANTIMALARIAL ACTIVITY OF *Canarium odontophyllum* "DABAI" ON *Plasmodium falciparum*

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Background:
Canarium odontophyllum is locally known as 'Dabai' or 'Sibu Olive' among Sarawakian. This plant comes from family of *Burseracheae* which can be found mainly in the tropical rainforest in Sarawak. Previous study showed methanolic extract of 'Dabai' leave poses high antimalarial activity against *Plasmodium berghei* NK65 through ex vivo. The present study was undertaken as continuity to identify the antimalarial properties of these leave extracts on *Plasmodium falciparum 3D7* through in vitro.

Methods:
Methanol, Acetone, Hexane and Aqueous leave extracts were tested in order to determine the antimalarial properties using cultured *Plasmodium falciparum* 3D7 in vitro. The growth inhibition was assessed through Plasmodium Lactate Dehydrogenase (pLDH) Assay.

Results:
The acetone extract possessed high inhibitory effect towards the growth of *P.falciparum* with 50% inhibition concentration of 226.6µg/ml. These followed by methanol extract with minimal difference in concentration of IC50 of ~269.4 µg/ml. However, both aqueous and hexane extract (IC50 of ~1442µg/ml) demonstrate low inhibitory effects with IC50 of ~1103µg/ml and ~1442µg/ml respectively.

Conclusion:
Therefore, acetone and methanol extracts were found to exhibit promising antimalarial activity against *P.falciparum*.

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ANTIVIRAL EFFECT OF STEM BARK AQUEOUS EXTRACT OF CANARIUM ODONTOPHYLLUM (DABAI) AGAINST DENV-2 IN VERO CELL LINE

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Background:
Dengue infection is an alarming vector-borne disease globally that can cause serious implications to patients. Over 40% of world population which represents more than 2.5 billion people are at risk from dengue. The number of cases is increasing in America, South East Asia and Western Pacific, for instance, in 2016, 100028 cases were recorded in Malaysia. Dengue infection is categorized based on sign and symptoms into three groups which are dengue fever (D), dengue with warning signs (DW) and severe dengue (SD). There are no specific antiviral treatment and vaccine for dengue which in turn worsens the situation. Natural products have been proposed as a source of antidengue drug development. For example, papaya leaves were proven to increase platelet number in dengue patients. Phytochemicals from natural products such as terpenoid, flavonoid, tannin and phenolic acid are believed to be responsible for anti-dengue effects. Thus this study was proposed to study antiviral effects of stem bark aqueous extract of *Canarium odontophyllum* (Dabai) against dengue virus (DENV)-2 in Vero cell line.

Methods:
Cytotoxicity of the extract was first determined using concentrations of 12.5-200 µg/ml on Vero cells. The 50% cytotoxic concentration (CC_{50}) was obtained using dose-response curve. The antiviral effects of the extract were studied using plaque reduction assay at concentrations below CC_{50}. Vero cells at 80% confluency were infected with DENV-2 at MOI 0.2 for 1 h at 37°C, 5% CO_2. The medium was removed and cells were washed with phosphate-buffered saline (PBS). Fresh medium containing extract at designated concentrations was mixed with CMC agar (final concentration = 2%, w/v) and then added to cells. The cells were then further incubated at 37^0C, 5% CO_2 for 7 days. Plaque number formed in treated wells is compared with that in untreated, control wells.

Conclusion:
In conclusion, *Canarium odontophyllum* (Dabai) aqueous extract is believed to possess anti-DENV-2 effects.

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ANTIFUNGAL EFFECTS OF LEMONGRASS AND EUCALYPTUS ESSENTIAL OILS ON FUNGI CAUSING NAIL INFECTION

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Background:
The principal causes of human nail infections include the dermatophyte *Trichophyton rubrum* and the mould *Aspergillus niger*, both of which appear to be causative fungal pathogens in an increasing number of cases. The current treatment for fungal nail infections does not eradicate the causative fungi permanently resulting in high chances of recurrence while resistance to antifungal agents has also increased. This study aimed to evaluate the in vitro antifungal activity of lemongrass and eucalyptus essential oils as natural product alternatives which may be more effective therapeutically.

Methods:
Two species of fungi, *Tricophyton rubrum* and *Aspergillus niger* were tested with commercially prepared essential oils using a disc diffusion assay to evaluate the inhibition zones and a microdilution assay to determine the Minimum Inhibitory Concentration (MIC).

Results:
Eucalyptus essential oil inhibited *Trichophyton rubrum* at the lowest concentration of 50% while the growth of *Aspergillus niger* was inhibited at 50% concentration in the disk diffusion assay. Lemongrass essential oil showed zone of inhibition towards only one of the fungi which is *Tricophyton rubrum* at 100% oil concentration. For the MIC determination, eucalyptus and lemongrass essential oils inhibited *Trichophyton rubrum* at concentrations of < 0.78% and 6.25% respectively while for *Aspergillus niger*, the fungus was inhibited only by eucalyptus essential oil at a concentration of 50%.

Conclusion:
This study showed that eucalyptus and lemongrass essential oils have antifungal activity towards *Tricophyton rubrum* and *Aspergillus niger* and may be used as an alternative treatment for nail infections in the future.

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CHARACTERISTICS AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *SALMONELLA* SEROTYPE ISOLATED FROM RAW CHICKEN AND MEAT CONTACT SURFACES

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Background:
Many cases of foodborne diseases are mostly associated with consumption of contaminated poultry meat. One of the major foodborne diseases globally is caused by salmonellosis. Due to high sources of protein, B complex vitamin and minerals, poultry meat is preferable environment for their growth. Evisceration step during poultry meat production is also one of the main sources of contamination. High prevalence of multi-drug resistant strains of *Salmonella* was detected in the poultry environment in Selangor. When food animals are slaughtered and processed, the resistant bacteria can contaminate the meat or other animal products. Increasing number of multidrug-resistant bacteria, consequently, cause in increased morbidity and mortality in cases.

Methods:
This study was carried out to determine the *Salmonella* spp., *Salmonella enteritidis* and *Salmonella typhimurium* isolated from raw chicken and meat contact surfaces by PCR method using the specific primers. To analyse the antibiotic susceptibility profile of the *Salmonella* serotype isolated by using disk diffusion method towards selected antibiotics. The isolates were molecularly identified for the presence of antibiotic resistance genes by using the PCR method.

Results:
PCR method was used to identify the *Salmonella* spp. serotype. From 7 samples isolated those were positive for *Salmonella*, the occurrence of *Salmonella* spp., *Salmonella enteritidis* and *Salmonella typhimurium* were 100% (n=7), 0% (n=0) and 0% (n=0) respectively. The antibiogram testing revealed there was multi-drug resistance among *Salmonella* spp. All the 7 isolates were resistance to erythromycin, chloramphenicol, tetracycline, penicillin and sulfamethoxazoletrimethoprim with 0 mm, 4 mm, 0 mm, 0 mm and 8 mm of zone of inhibition respectively. A PCR method was also used to detect the presence of antibiotic resistance genes in the isolated *Salmonella* that confer resistance to the antibiotics used during antibiogram testing.

Conclusion:
Our results demonstrated that the isolated *Salmonella* could be a source of multiple antimicrobial-resistant of *Salmonella* and may contribute to a major health concern in Malaysia. Knowledge of variability in resistance pattern of *Salmonella* and sources of *Salmonella* may provide valuable added information for research, risk management and public health strategies.

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THE SYNERGISTIC EFFECT OF PENICILLIN AND METHICILLIN WITH *Clarias batrachus* MUCUS AGAINST GRAM NEGATIVE BACTERIA

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Background:
Resistance towards antibiotics occurs due to the excessive and prolongs usage of antibiotics as well as error in prescription that leads to the declined usage of existing antibiotics in the market. So, a strategy or alternative to allow reuse of the old antibiotics is by combining the antibiotic with natural sources in order to increase the efficiency of the antibiotic in treatment. Therefore, this study is conducted to determine the synergistic effect of Penicillin and Methicillin with *Clarias batrachus* mucus against *Escherichia coli* and *Pseudomonas aeruginosa.*

Methods:
In this study, the determination of antibacterial activity of *C.batrachus* was carried out by using the spectrophotometric method. Then, the minimum inhibitory concentration (MIC) of *C.batrachus* mucus and both antibiotics against *E.coli* and *P.aeruginosa* were determined by using broth microdilution assay in 96 wells microtitre plate. While, for the interaction of both antibiotics with the *C.batrachus* mucus was evaluated by using the Checkerboard assay and being analyzed by calculation of Fractional Inhibitory Concentration (FIC). Besides, the outer membrane changes for both gram-negative bacteria when tested with the mucus were observed under Scanning Electron Microscope.

Results:
From the study, it's found that the *C.batrachus* mucus showed the antibacterial activity towards *E.coli* and *P.aeruginosa*. The value of minimum inhibitory concentration (MIC) for *C.batrachus* mucus when tested against *E.coli* and *P.aeruginosa* was greater than 40mg/ml. While, when Penicillin and Methicillin were tested against *P.aeruginosa*, the MIC for both antibiotics was greater than 4mg/ml. Besides, the MIC for Penicillin and Methicillin against *E.coli* was detected at 125µg/ml and 2mg/ml respectively. The interaction study showed that there was no synergistic effect between *C.batrachus* mucus with Penicillin or Methicillin against *P.aeruginosa*. However, the combination study showed better effects since the concentration of Penicillin and Methicillin had reduced significantly against *E.coli* compared to Penicillin and Methicillin alone. Observation under SEM revealed that there were changes to the outer membrane of *E.coli* and *P.aeruginosa* when tested with *Clarias batrachus* mucus.

Conclusion:
In conclusion, the *C.batrachus* mucus enhanced the activity of Penicillin and Methicillin towards gram-negative bacteria, *E.coli* and *P.aeruginosa*.

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IN VITRO STUDY OF CYSTICIDAL EFFECT OF THE ANTIMICROBIAL AGENTS ON *ACANTHAMOEBA* SPP. FROM CLINICAL AND ENVIRONMENTAL ISOLATES

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Background:
Acanthamoeba keratitis (AK) is a type of serious corneal infection that may result in severe inflammatory reaction and visual loss. AK is mostly associated with the use of contact lenses due to poor hygiene status among the contact lens wearer. Nowadays, resistance of the *Acanthamoeba* cysts towards antimicrobial agents increases from time to time. Therefore, this study was performed to evaluate the effectiveness of the therapeutic dose and the minimum cysticidal concentration (MCC) of the antimicrobial agents; 0.02% chlorhexidine digluconate and 0.01% propamidine isethionate on clinical isolates (HUKM 74 and HS 62) and environmental isolates (SG 7 and OT 3).

Methods:
Serial doubling dilution for chlorhexidine digluconate from 200 µg/ml to 0.0977 µg/ml and propamidine isethionate from 1000 µg/ml to 0.4883 µg/ml were performed to obtain their MCC. After the exposure of the cysts to each concentrations of the antimicrobe for 24 hours, the suspension was washed three times followed by centrifugation process. The cysts deposits then were cultured on nonnutrient agar plate containing *Escherichia coli*. The effectiveness of the therapeutic dose without doubling dilution were determined based on the presence of the trophozoites from the cysts after incubation period. The presence of trophozoites indicates the ineffectiveness of the agent against the *Acanthamoeba* cysts. Meanwhile, the MCC of each antimirobial agent were recorded after 14 days of microscopic examination.

Results:
Chlorhexidine digluconate and propamidine isethionate were successfully found to be effective against all isolates of *Acanthamoeba* cysts at therapeutic dose. The mean value of MCC for the chlorhexidine and propamidine isethionate was 46.88 ± 6.25 µg/ml and 406.25 ± 157.29 µg/ml, respectively. The environmental isolates show higher MCC than clinical isolates when treated with propamidine isethionate meanwhile the MCC of the clinical isolates were slightly higher than environmental isolates when exposed with chlorhexidine digluconate.

Conclusion:
So from this study, it can be concluded that both antimicrobial agents chlorhexidine and propamidine isethionate were effective against all strains tested and suitable for the treatment of AK.

KEY WORDS:

Acanthamoeba, in vitro-sensitivity test, chlorhexidine digluconate, propamidine isethionate

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Cloning of Influenza *M2e-NP* Fusion Gene into *pRSET B* Bacterial Expression Vector

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Background:
Despite the presence of anti-influenza vaccine and drugs, influenza A is still causing high mortality and mobility in humans. The current vaccine only targets circulating virus strains and therefore only offers specific protection against those strains. A universal vaccine is needed for protection against many unpredicted strains. As a conserved antigen, the extracellular domain of influenza A M2 protein (M2e) is able to elicit immunity that prevents infections by all influenza A subtypes. In order to increase its efficacy, nucleoprotein (NP) of influenza A with prominent adjuvantivity is used to present M2e. This study attempted to fuse M2e to the N-terminus of NP using fusion PCR.

Methods:
cDNA was generated using random primer, reverse transcriptase (M-MLV) and total RNA extracted from H5N1-infected MDCK cells. M2e and NP genes were amplified using GoTaq HotStart polymerase and gene-specific primers. The optimum annealing temperatures for M2e and NP gene amplication were 55ºC and 71ºC respectively, respectively. The PCR products were ligated to yT&A vector at 37 ºC for 18 h. About 5 µl of ligation mixture was transformed into $CaCl_2$competent *E. coli* strain TOP 10. Bacterial colonies were screened using PCR for insert. Plasmids, yTA-M2e and yTA-NP were extracted and used as templates in fusion PCR. M2e and NP genes were fused using DyNAzyme DNA polymerase, purified and digested with *Bam*HI and *Hind*III. The digested product was then cloned into *pRSET* B vector excised with *Bam*HI and *Hind*III. The recombinant plasmid was then verified using DNA sequencing.

Results:
Gel electrophoresis showed that M2e and NP were successfully amplified using cDNA. Ten colonies were selected randomly from each yTA-M2e and yTA-NP plates. The PCR screening results indicated that both of genes were inserted into the vector. On 1% agarose gel, a DNA band of 1.6 kb was observed. The fusion product and pRSET B were digested using *Bam*HI and *Hind*III and then ligated using T4 DNA ligase. DNA sequencing result proved that pRM2e-NP carried M2e and NP sequences from H5N1 (A/chicken/Malaysia/5858/2004(H5N1)).

Conclusion:
Fusion of M2e and NP genes is possible which in turn can be used in developing M2e-NP universal influenza vaccine.

Keywords: fusion PCR; influenza A; M2e; Nucleoprotein; vaccine.

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Development of Alternative Culture Media Using White Rice and Spinach

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Introduction:
Basic medium such as nutrient agar and nutrient broth are used as cultivation media for most of living microorganisms. A few studies had developed culture media as alternative to the available basic medium using their own local products which working towards cost-effective development. White rice contains 90% of carbohydrate that can be used as source of energy for metabolism of organisms. Meanwhile, spinach is an excellent source of macro- and micronutrients for growth of organisms.

Objective:
This study focused on designing alternative media using local products of spinach and white rice for growth of S. aureus, B. subtilis, Salmonella sp. and E. coli. Effectiveness of the media to support the bacteria growth was determined by the colony forming units (CFU) counting technique.

Methods:
The alternative media containing spinach and white rice extracts were prepared at different ratios and cultured with the *S. aureus*, *B. subtilis, Salmonella sp. and E. coli*. The mixtures were then incubated for overnight at 37^0C with 5% CO_2 and concentrations of the bacteria following the incubation process were determined using the CFU counting technique.

Results:
Increase of carbohydrate content was found to slightly elevate the growth of *E. coli* and *Salmonella*. Meanwhile, increase of spinach concentration was observed to support growth of *Salmonella*, *S. aureus* and *B. subtilis* significantly.

Conclusion:
White rice and spinach have great potential to be used as source of carbohydrate and other nutrients in developing basic bacterial culture media.

Keywords: Alternative culture media, *E. coli, Salmonella sp*., *S. aureus, B. subtilis*, spinach and white rice.

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Investigation of *Trichuris Trichiura* from Soil Samples at Kampung Orang Asli Sungai Lalang Baru, Ulu Semenyih, Selangor.

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Backgrounds:
For decades soil-transmitted helminthes (STHs) has been a great concern throughout the world among the poorest and the most deprived communities. Most of the Orang Asli in Malaysia are still living with poor sanitation facilities with multiple species of STH infection. Due to their poor environment condition, they are prone to both transmission and reinfection of STHs, especially from the contaminated soil which is the main reservoir for STHs. Hence, this study was carried out to investigate the presence of *Trichuris trichiura* in the soil samples surrounding Orang Asli village located at Sungai Lalang Baru, Ulu Semenyih, Selangor.

Methods:
The soil samples were collected from Kampung Asli Sungai Lalang. The samples were collection between June till November 2017. The soil samples collected were sealed in a plastic bag and preserved in the freezer. Approximately 200-250 gram of soil samples were collected from a depth of about 4-6inch. Then, sedimentation technique was used to recover the *Trichuris trichiura egg* from the soil samples. The samples were view under 40x using light microscope for confirmation.

Results:
Total soil samples collected were 40 samples, in which 6 out of it were detected positive with *Trichuris trichiura* eggs. The overall percentage of *Trichuris trichiura* eggs from the soil samples collected from Kg Asli Sungai Lalang was 15%. Sampling area near pond, surrounding housing area and nearby road showed presence of *Trichuris trichiura eggs*.

Conclusion:
This finding showed that several land areas in Kg.Asli Sg.Lalang to be contaminated with *Trichuris trichiura.* This could lead to transmission of *Trichuris trichiura* infection to the villages. The future study is to explore on the role of domestic animals in transmitting *Trichuris trichiurai* infection to the villages at Kg.Asli.Sg,.Lalang, Ulu Semenyih, Selangor.

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DISTRIBUTION OF CHEMOKINE RECEPTOR 2 POLYMORPHISM (CCR2-V64I) IN MALAYSIAN HIV-1 INFECTED INDIVIDUALS

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Background:
Chemokine receptor-2 (CCR2) is a co-receptor for the entry of human immunodeficiency virus-1 (HIV1) into the target cells. Genetic polymorphism of CCR2 human chemokine receptors have been associated with resistance during HIV-1 infection and disease progression. The protective effect of mutant alleles at these loci has important implications in AIDS pathogenesis. The G-to-A transition was found to be occurred at position 190 characterizes the CCR2-V64I mutation, causing valine to isoleucine substitution. This polymorphism has been identified to be associated with delayed AIDS progression and protects against HIV-1 infection. However, there is limited report on distribution of this polymorphism in Malaysian population. Therefore, this study aimed to determine the existence and distribution of CCR2-V64I mutation among HIV-1 infected patients.

Method:
Extracted DNAs from HIV-1 infected individuals include 59 Malay, 58 Chinese and 33 Indian have been analysed for the presence of CCR2-V64I using Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP). The amplicons have been visualized through 2.0% of agarose gel electrophoresis. Genotypes of homozygous wild type, heterozygous and homozygous mutant allele have been collected among the samples. Fisher Exact test was used to determine the Hardy-Weinberg equilibrium and identify the association between genotypes and ethnics.

Results:
Out of 150, 140 samples were successfully determined their genotype. There were 65 homozygous wild type includes 22 Malay, 25 Chinese and 18 Indian. Meanwhile for heterozygous, there were 32 Malay, 26 Chinese and 14 Indian. There were only 3 homozygous recessive includes 2 Malay and 1 Chinese. The genotype frequency of CCR2-V64I was not found in the Hardy-Weinberg equilibrium. Furthermore, the fisher exact test showed no association between genotypes and ethnics with p value more than 0.05. In addition, there is also no significant different between control population and case population. In this study, 51.4% of the HIV-1 individuals were identified as heterozygous that potentially have a delayed HIV progression to AIDS.

Conclusion:
As conclusion, all the three genotypes were found in the Malaysian HIV-1 population. CCR2-V64I may have potential in HIV-1 progression and therefore, further studies are needed using biomarker such as CD4 level to support this statement.

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Expression, Purification and Characterisation of the P-domain of *Marcobrachium rosenbergii Nodavirus*

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Background:
Giant freshwater prawn, *Macrobrachium rosenbergii*, is the most economically significant reared aquatic invertebrate in sub-tropical countries. However, this large-scale cultured prawn is susceptible to *Macrobrachium rosenbergii nodavirus* (*Mr*NV) infection which leads to white tail disease (WTD). Clinical signs of WTD include lethargy, milky whitish coloration of the abdominal muscle and degeneration of the telson and uropods. The disease causes great economical loses due to its 100% mortality rate in post-larvae of prawn. The virion of *Mr*NV contains two segmented RNA molecules which encode the RNA dependent RNA polymerase (RdRp) and the capsid protein (*Mr*NV-CP). The full length *Mr*NV-CP consists of 371 amino acid residues which can be divided into 2 major domains: shell (S) domain and protruding (P) domain, ranging from amino acids 1-250 and 251-371, respectively. The P-domain (C-terminal region) has been shown to be involved in host cell attachment and internalisation. Structural study of *Mr*NV virus like particles (VLPs) using cryo-electron microscopy shows that the P-domain of *Mr*NV-CP assembled into dimeric protruding spikes. However, its 3D structure remains unavailable.

Methods:
The P-domain of *Mr*NV-CP was expressed using an *Escherichia coli* expression system and was purified with immobilised metal affinity chromatography (IMAC) and size exclusion chromatography (SEC). The purified protein was then characterised using circular dichroism (CD) assay and dynamic light scattering (DLS).

Results:
The P-domain of *Mr*NV-CP was expressed at 30 ºC as soluble protein with a molecular mass of ~18 kDa as estimated using SDS-PAGE. In IMAC chromatography, the protein was eluted using 100mM imidazole and further purified to ~ 90% purify with SEC. By comparing the chromatogram of protein markers, the P-domain of MrNV-CP is believed to assemble to form dimers. CD analysis showed that the P-domain of *Mr*NV-CP contains 67.9% beta structure and 32.1% of turn when analysed using the Reed's reference. DLS analysis showed that the diameter of the P-domain of *Mr*NV-CP is ~8 nm.

Conclusion:
The recombinant P-domain of *Mr*NV-CP was successfully expressed, purified and characterised. The purity of the protein is about 90%, which merit further analysis such as protein X-ray crystallography to solve its 3D structure.

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INVESTIGATION OF CHEMOKINE RECEPTOR CCR5-Δ32 AND SINGLE NUCLEOTIDE POLYMORPHISM R223Q IN MALAYSIA'S HIV-1 INFECTED INDIVIDUALS

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Background:
Chemokine receptor CCR5 acts as a co-receptor that mediates human immunodeficiency virus type-1 (HIV-1) entry into susceptible cells. A 32bp deletion (Δ32) in CCR5 gene renders the receptor to be non-functioning, hence, is associated with reduced risk of contraction of HIV-1 and delay in the progression of AIDS. Meanwhile, R223Q is A/G single nucleotide polymorphism (SNP) of CCR5 gene changing arginine (R) to glutamine (Q) which could also affects the receptor function. The aim of this study was to assess the frequency of CCR5-Δ32 and to genotype R223Q SNP among HIV-1 infected population in Malaysia.

Method:

Genotyping of CCR5 polymorphism (Δ32 and R223Q) was carried out using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP). Extracted DNA from 150 HIV-1 patients in Malaysia, including 59 Malays, 59 Chinese, and 32 Indians, were subjected to amplification of CCR5-Δ32 and R223Q regions. PCR products were visualised through 2% agarose gel electrophoresis to confirm the presence of amplicon and subsequently digested using TspRI restriction enzyme. Hardy-Weinberg equilibrium was tested for the two CCR5 polymorphisms among the three major ethnic groups in the population. Additionally, association between ethnic and genotype was examined using chi square analysis.

Results:

Out of 150 samples, there were 2 HIV-1 samples which did not show amplification after PCR, thus, only 148 samples were analysed. There were 147 samples for homozygous wild type while one sample was heterozygous for Δ32. Meanwhile, homozygous genotype of R223Q was observed in 146 wild type and 2 of them were heterozygous. Therefore, allele frequency for homozygous wild type and heterozygous for Δ32 were 99.7% and 0.3% respectively. Meanwhile, allele frequency for homozygous wild type and heterozygous for R223Q were 99.3% and 0.7% respectively. The genotypic frequency for Δ32 and R223Q were not found to be in Hardy-Weinberg equilibrium. Furthermore, Pearson's chi square test revealed no significant association between the three major ethnics in Malaysia and the CCR5 polymorphisms.

Conclusion:

Our data suggested both polymorphisms (Δ32 and R223Q) cannot be considered as a measure of genetic susceptibility toward HIV progression as it was found to be very rare mutations in Malaysian HIV population. However, it could be due to the small sample size too.

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DEVELOPMENT OF IN SITU HYBRIDIZATION ASSAY FOR THE DETECTION OF ENTEROVIRUS D68 RNA IN FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUE

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Background:
Enterovirus D68 (EV-D68) is a single-stranded positive sense RNA virus belongs to the enterovirus D species within the *Picornaviridae* family. EV-D68 is a common causative agent of viral respiratory infection. Recently, EV-D68 has emerged as one of the important neurotropic enteroviruses that causes severe respiratory disease associated with acute flaccid myelitis, especially in young children. Up to date, the pathogenesis of EV-D68 infection is still unclear, partly due to the lack of specific detection tools that allow localization of EV-D68 infection at the cellular/tissue level. In this study, we have developed an *in situ* hybridization (ISH) assay using digoxigenin-labeled DNA probe for the detection of EV-D68 RNA in formalin-fixed and paraffin-embedded (FFPE) tissues.

Methods:
An approximately 620-bp PCR fragment covering EV-D68 VP1 region was generated from the extracted viral RNA and cloned into a vector using standard molecular cloning procedure. The extracted plasmid containing the correct insert was sent for sequencing before used as a template to produce digoxigeninlabeled DNA probes using PCR method. ISH assay was then performed to test the specificity of the synthesized probes on positive controls (EV-D68 infected RD cell blocks) and negative controls (mockinfected RD cell blocks, non-EVD68 infected cell blocks, EV-A71 infected mouse tissues and normal human tissues).

Results and Discussion:
All the positive and negative controls showed expected results. The positive staining was confined only to the cytoplasm, while the nucleus showed negative results. No non-specific staining and background staining were observed in all control slides. These results showed that the synthesized digoxigenin-labeled probe was specific to EV-D68 RNA and did not hybridize to other enteroviruses, mouse and human RNA.

Conclusion:
In conclusion, this newly developed ISH assay could serve as a useful tool for detection of EV-D68 infection in clinical specimens, and pathogenesis study.

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Detection of Leptospira DNA among febrile patients without dengue fever

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Background:
Leptospirosis is an emerging zoonosis that affects people in tropical and subtropical regions. It is a febrile disease that is endemic in Malaysia, with the first documented human leptospirosis case reported in 1925. The severe form of leptospirosis, also known as Weil's disease, can cause death in humans. This disease is transmitted by direct contact with the infected host or indirect contact with the soil or water that is contaminated by the infected animal's urine. Early clinical symptoms of leptospirosis are non-specific and its classical symptoms are identical to other common tropical diseases such as malaria and dengue fever. Thus, this causes a diagnostic challenge and, would eventually leads to the misdiagnosis and the underreporting of the leptospirosis cases. The objective of this study is to determine the prevalence of leptospirosis in febrile patients without dengue fever. The hypothesis is that *Leptospira* might be the causative agent of infection in febrile patients with the primary suspicion of dengue fever.

Method:
A total of 246 archived blood clots of non-dengue febrile volunteers recruited from district health clinics around the state of Selangor were used. Blood clots were subjected to DNA extraction. The extracted DNA was examined for *Leptospira*-specific DNA using semi-nested polymerase chain reaction (PCR).

Results:
Out of the total number of febrile volunteers (n=246), *Leptospira*-specific DNA was detected from five (n=5) volunteers, resulting in a positivity rate of 2.03%.

Conclusion:
From the findings of this study, *Leptospira* sp. was found as a pathogen that causes infection among febrile patients without dengue fever. It is therefore important to differentiate leptospirosis and dengue fever because early administration of antibiotics will help in improving clinical outcome of leptospirosis significantly.