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ISOLATION OF *ACANTHAMOEBA* SPP. FROM CONTACT LENS IMPEDIMENTA OF HEALTHY CONTACT LENS WEARERS IN SHAH ALAM

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Background:
Acanthamoeba spp. is a type of free-living-amoeba (FLA) that is omnipresent in nature but it can also be the contaminant of contact lens along with its paraphernalia, tap water, chlorinated swimming pool water and also air-conditioning system. With the upsurge in the number of contact lens wearers in Malaysia as well as low compliance towards contact lens maintenance, it is pertinent that this parasite could inhabits contact lens impedimenta and subsequently be a potential conduit to loss of vision to the users. Hence, this study is conducted prior to isolating *Acanthamoeba* spp. from contact lens, contact lens solution and contact lens case of healthy contact lens wearers in Shah Alam.

Method:
In vitro cultivation technique was implemented in the study where the aforementioned specimens were swabbed with sterile cotton swabs and cultured on non-nutrient agars supplemented with thermalinactivated *Escherichia coli* and the agars were incubated for 14 days and were observed daily. Then, positive samples were stained using iodine stain, methylene blue stain as well as trichrome stain and were observed using compound microscope. A questionnaire was distributed to the respondents prior in assessing the factors that engender the contamination by *Acanthamoeba* spp.

Results:
Of the 150 samples from 50 respondents, 20% of contact lens samples, 16% of contact lens solution samples, and 22% of contact lens case samples were positive for *Acanthamoeba* spp. It was also found that 100% of the positive contact lens samples were extended wear soft contact lens type. The isolates are predominantly Group II and are medically important. Fail to soak contact lens with enzymatic tablet (p=0.002) was found to be the paramount non-compliance practice that engender contamination by *Acanthamoeba* spp. followed by rinsing contact lens with saline (p=0.007).

Conclusion:
As contact lens is gaining popularity among the community and the number of contact lens wearer in Malaysia is escalated, public health awareness and education to the community should be intervened to enhance the level of compliance towards contact lens maintenance and expectantly to repress contact lens related microbial keratitis, particularly *Acanthamoeba* keratitis.

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EFFECTIVENESS OF CREAM REPELLENT CONTAINING MIXTURE OF ESSENTIAL OILS AGAINST *Blattella germanica* (DICTYOPTERA: BLATTELLIDAE) AND *Musca domestica* (DIPTERA: MUSCIDAE)

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Background:
Cockroach *Blattella germanica* and house fly *Musca domestica* are house pests that function as vectors in transmission of various medically important pathogens. One way to control and reduce disturbances of these pests is by using repellent. Development of natural based repellent may become an alternative to reduce the use of synthetic repellent that poses harmful risk to human's health and environment.

Methods:
Four different cream repellent formulations containing mixture of essential oils from *Litsea elliptica, Piper aduncum* and *Piper sarmentosum* were prepared based on their ED_{90} obtained from previous studies. The repellent properties were investigated in the laboratory testing and compared with the positive control.

Results:
In the laboratory testing of *B. germanica*, all cream repellents provided over 90.00% repellency at 1 hour post-application up until 6 hours, with the exception of cream repellent containing mixture of *P. aduncum* and *P. sarmentosum* essential oils which drop to 71.68% repellency after 2 hours. At 48 hours post-application, only cream repellent containing the mixture of all essential oils provided over 90% repellency while the other cream repellents have decreased repellency effect. Besides, the cream repellent containing the mixture of all essential oils provided a higher repellency effect (98.33%) as compared to the positive control, naphthalene (93.33%) at 48 hours post-application. In the laboratory testing of *M. domestica*, at 4 hours post-application, cream repellent containing mixtures of *L. elliptica* and *P. aduncum* essential oils only provided 64.60% repellency, and the cream repellent containing mixture of *L. elliptica* and P. *sarmentosum* essential oils provided 87.77% repellency, while cream repellent with mixture of *P. aduncum* and *P. sarmentsoum* provided 91.67% repellency. The highest repellency effect was shown by the cream repellent containing the mixture of all essential oils (93.93%). However, all cream repellents show lower repellency effects when compared to the positive control, 5% citronella cream that provided 100% repellency at 4 hours post application.

Conclusion:
In conclusion, all cream repellents formulations have good potential to be developed into a repellent product against *Blattella germanica* and *Musca domestica*. Combination of several essential oils may increase the efficacy of repellent products.

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ISOLATION AND CHARACTERISATION OF BACTERIOPHAGES AS POTENTIAL BIOCONTROL AGENT AGAINST PATHOGENIC *PANTOEA* SPP.

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Background:
In between September to December 2016, a major outbreak of bacterial leaf blight (BLB) disease occurred in the paddy field of Sekinchan, Selangor. This BLB outbreak was the worst in the last 30 years, reducing the yield up to 70% where 4,440 ha of paddy field was affected, causing losses of RM 5 million to local farmers. 16S rRNA sequencing of bacteria isolated from the diseased paddy plants during the outbreak suggests *Pantoea* spp. are the causative agents of this BLB. *Pantoea* is a rod-shaped Gram-negative bacterium belongs to Enterobacteriaceae family. Emergence of *Pantoea*-associated BLB cases of paddy plants have been documented in India, China, Korea, Russia and West Africa recently. Yet, limited studies have been directed to control this *Pantoea*-associated BLB. To date, the application of bacteriophages for disease control is a fast expanding area of crops protection. Therefore, we aim to isolate bacteriophages as potential biocontrol agents of phytopathogenic *Pantoea* spp.

Methods:
Soil samples from paddy fields were collected in Sekinchan and Permatang Pauh. These samples were enriched with *Pantoea* spp. to promote growth of *Pantoea*-specific bacteriophages. Double agar overlay plaque assay and host range analyses of the bacteriophage isolates were conducted. Purified bacteriophage genomes were treated with DNase and RNase for nucleic acid typing. Subsequently, restriction fragment length polymorphisms were analyzed to determine the no. of phages obtained.

Results:
Our virulence tests on rice cultivars showed that *Pantoea* spp caused the BLB disease. In this study, a total of six lytic dsDNA-bacteriophages were isolated. Among them, bacteriophage WP57 showed the widest host range that lysed *P. septica*, *P. dispersa*, and a novel *Pantoea* spp. Bacteriophages JCL2, C7D1 and C7F2E killed two different species of *Pantoea*. Lastly, bacteriophages J8CS and B7C were found to specifically lyse *P. septica* and the novel *Pantoea* spp., respectively.

Conclusion:
Taken together, we have isolated a cocktail of six closely-related bacteriophages that may target different docking sites for effective control of their pathogenic hosts. As a biocontrol agent, bacteriophages may provide a 'green' technology for controlling BLB disease in rice, which may serve as a model for developing bacteriophage-based biocontrol in other crops as well.

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THE ABILITY OF COMMERCIALLY AVAILABLE CONTACT LENS DISINFECTING SOLUTION IN EXHIBITING ANTI-ACANTHAMOEBA EFFECT ON CYSTS OF CLINICAL AND ENVIRONMENTAL ISOLATES

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Background:
The increased risk of ocular infection and diseases especially *Acanthamoeba* keratitis among contact lens wearers has been attributed to the increasing trend of contact lens wear over the years. Multipurpose solutions of different brands and types which are available commercially are the first line defence in hindering the occurrence of ocular infection and diseases caused by microorganisms. Hence, this study was done to assess the ability of commercially available contact lens disinfecting solutions in restricting the excystment of *Acanthamoeba* cyst of clinical and environmental isolates based on the recommended time of soaking; 4 and 6 hours and overnight soaking for 8 hours.

Methods:
Contact lens disinfecting solution of the brand Polylab® Multipurpose Solution, Opticare Multimate™ and Oxysept® were tested with *Acanthamoeba* of clinical isolates; HUKM 74 and HS 62 together with environmental isolates; SG 7 and OT 3. These isolates were subcultured onto non-nutrient agar (NNA) plates for 10 to 14 days and the cyst suspension obtained from each of the isolates was mixed with the disinfecting solutions separately. The mixture was then dropped onto NNA plates seeded with heat-killed *Escherichia coli* and incubated at 30ºC (±4ºC). They were observed under an inverted microscope for 14 days to detect the presence of trophozoite which signifies the inefficiency of the disinfecting solution used.

Results:
Polylab® Multipurpose Solution and Opticare Multimate™ were unable to inhibit the growth of all *Acanthamoeba* isolates with evidence of trophozoites; however Oxysept® demonstrated its anti-*Acanthamoeba* effect, with no trophozoites present.

Conclusion:
This proves that Polylab® Multipurpose Solution dan Opticare Multimate™ are not suitable to be used by contact lens wearers to prevent *Acanthamoeba* keratitis.

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Investigating the Expression Level of Pathogen Recognition Receptors (PRRs) in *Helicobacter pylori*-Infected Macrophages and Cloning of Mincle into a Lentiviral Vector System

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Background:
Helicobacter pylori is a Gram-negative bacterium recognized as the most common cause of gastric cancers. Once infected, the host is often unable to completely eradicate the infection, which leads to longterm persistence, indicating the ability of *H. pylori* to evade immune detection. Recent studies suggested that several pattern recognition receptors (PRRs) are involved in *H. pylori* evasion of host immune defense. Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) are two families of PRRs that recognize their respective ligands on pathogen cell surface or internalized nucleic acids. This study thus aims to investigate the changes in expression of TLRs and CLRs in immune cells upon *H. pylori* infection. This is followed by the cloning of *Macrophage inducible C-type lectin* (*Mincle*) into a Lentivirus vector system to further elucidate the role of this CLR in *H. pylori* infection.

Methods:
Human monocytic macrophage cells THP-1 were infected with *H. pylori* strains J99 and SS1 at a multiplicity of infection (MOI) of 10 for 16 hours. RNA extraction, cDNA synthesis and qPCR were then performed to identify the changes in expression levels of 10 TLR targets (TLRs 1 to 10) and 3 CLR targets (Mincle, Dectin-1 and Dectin-2). Out of the 13 targets, *Mincle* gene was selected and amplified using high fidelity PCR. Double restriction enzyme (RE) digestion of *Mincle* amplicons and pLVX-Puro plasmid was performed followed by ligation and transformation into DH5α competent cells. Presence of positive clones after ampicillin selection was confirmed by diagnostic RE digestion and Sanger sequencing.

Results:
An upregulation in relative expression was observed in most TLRs and CLRs upon exposure to *H. pylori*. Amongst the 13 targets studied, the most significant increase in expression level was observed in *Mincle*. Subsequent cloning of the *Mincle* gene into pLVX-Puro plasmid vector was successful, producing a pLVX-Puro-Mincle construct.

Conclusion:
Our study identified *Mincle* as the most highly upregulated PRR in immunity to *H. pylori* infection. A successful cloning of this gene into Lentivirus system would pave a way for further investigation of its role in bacterial recognition and the strategies utilized by *H. pylori* to evade immune detection.

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POTENTIAL ANTILEPTOSPIRAL ACTIVITY OF *Canarium odontophyllum*

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Background:
Leptospirosis is a re-emerging zoonotic disease which can spread from an affected animal to human which caused by bacteria from genus Leptospira that pathogenic to human by the direct contact with the urine of infected animal whether through ingestion or wound. Between 2011 until 2016, Ministry of Health Malaysia reported that there are 31,771 cases of Leptospirosis that lead to 396 deaths in Malaysia. Numerous attempts have been made to control the disease by chemoprophylaxis but only showed limited success. *Canarium odontophyllum* or known as dabai are tree that can be found in tropical rainforest in Sarawak. Leaves extract from this tree possesses antibacterial activity. This study investigates the antileptospiral potential of *Canarium odontophyllum* crude leaves extract which is methanol, aqueous, acetone and hexane as antileptospiral agent.

Methods:
Antileptospiral activity of Canarium odontophyllum leaves extracts were tested against two serovar of pathogenic Leptospira which is *L.interrogans* serovar Bataviae and Javanica. The extract was tested in seven different concentrations by broth microdilution method to determine the minimum inhibition concentration (MIC) and obtain IC_{50} value through OD reading at 400nm. IC_{50} of most effective extract was incubated with Leptospira to observe and compared population changes under dark field microscope with untreated culture of Leptospira. On the other hand, IC_{50} of most effective extract also used to do the DNA damaging studies on Leptospira through gel electrophoresis of genomic DNA of treated Leptospira.

Results:
Most effective extract that exhibit antileptospiral activity towards both serovar were methanol extract. IC_{50} value which was obtained from the inhibition percentage curve for serovar Bataviae is 3.65 mg/ml and 4.5 mg/ml for serovar Javanica. Based on dark field microscope observation, the culture of Leptospira which were treated with IC_{50} value of extract for each serovar showed drastic changes of its population compared to untreated Leptospira culture for both serovar. There was no DNA damage exhibited by this extract towards both serovar since there is no DNA fragmentation on the gel.

Conclusion:
In conclusion, methanol leaves extract of *Canarium odontophyllum* has a potential to control Leptospirosis.

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DEVELOPMENT OF QUANTITATIVE REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION FOR THE DETECTION OF GETAH VIRUS

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Background

Getah virus (GETV), a mosquito-borne alphavirus, is an emerged equine pathogen causing recurrent outbreaks among racehorses in Japan and India. GETV infected horses showed symptoms varies from fever, lower limb oedema, swelling of the submandibular lymph nodes, urticarial and stiffness. The GETV infection in pigs caused foetal death and reproductive failure. Febrile illness has been reported in human patients tested positive with anti-GETV antibodies. GETV was first isolated in Malaysia in 1955. Antibodies against GETV have been detected in the local horses and pigs in Peninsular Malaysia since 1960s yet to date there has been no report of outbreak of the disease. This can be reasoned by the under-reporting attributed to the nature of GETV infection which causes mild diseases in various species as well as under-diagnosis due to unavailability of proper detection tools. This suggested the importance of surveillance for this virus in the region and thus the need of a tool for specific detection of GETV. Hence, in this study, we intended to develop a quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assay for the rapid detection and quantification of GETV.

Methodology

Two sets of GETV specific primers and Taqman probes were designed from the conserved region of nsP1 and nsP2 genes, respectively, based on the multiple sequence alignment of complete GETV genomes (n=25) obtained from Genbank. The GETV RNA standard was prepared using RNA *invitro*-transcribed from plasmid constructs containing the target sequences of GETV nsP1 and nsP2 genes, respectively. The RNA concentration was measured with spectrophotometer and the RNA copy number was calculated using ENDMEMO software available online (http://endmemo.com/bio/dnacopynum.php). The detection limit, coverage and cross-reactivity of the primers and probes were evaluated. The developed qRT-PCR assay was further evaluated for sensitivity and specificity using the simulated clinical samples spiked with different concentration of GETV.

Results

The qRT-PCR assay using both GETV nsP1- and nsP2-targeting primers and probe showed good efficiency of 92.74% (R^2=0.996) and 99.24% (R^2=0.996), respectively. The detection limit of qRTPCR assay using GETV nsP1 and nsP2 primers/probe was determined to be 194 copies and 63 copies, respectively. Both nsP1 and nsP2 primers/probe detected all two GETV strains isolated from Malaysia (M2021 and B254). While the GETV nsP1 primers and probe showed cross-reactivity with Chikungunya virus, Sindbis virus and Dengue virus, the GETV nsP2 primers and probe showed no cross-reactivity with other closely related alphaviruses or flaviviruses tested in this study. The qRTPCR assay using the GETV nsP2 primers and probe showed sensitivity and specificity of 100% based on the evaluation performed on the simulated clinical samples (n=16).

Conclusion

The qRT-PCR assay developed in this study is useful for rapid detection and quantification of GETV.

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Investigation of *Ascaris Lumbricoides* from Soil Samples at Kampung Orang Asli Sungai Lalang Baru, Ulu Semenyih, Selangor

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Introduction:
Globally, it is estimated that over 1.5 billion people are infected with at least one species of soil-transmitted helminths (STHs). *Ascaris lumbricoides* is the "largest roundworm" reported and categorized under soil transmitted helminths (STHs). Presence of *Ascaris lumbricoides* has been reported in Malaysia among the rural and the indigenous community (Orang Asli) in Malaysia. This infection is transmitted via contaminated soil surrounding their houses and causes malnutrition and intestinal problems among the community, especially the children.

Objective:
To investigate presence of *Ascaris lumbricoides* in soil samples from Kampung Orang Asli Sungai Lalang Baru, Ulu Semenyih.

Methods:
Soil samples were collected from 5 areas surrounding the village and various soil types like sandy soil, loamy soil and clay soil were collected during rainy and dry season. Next, formalin-ethyl acetate sedimentation technique was used to isolate the parasite eggs from the soil samples. Positive soil samples were identified using light microscopy.

Results:
Presence of *Ascaris lumbricoides* eggs were detected from all the soil samples collected. It was found, loamy soil showed the highest presence of *Ascaris lumbricoides* and more eggs were detected during dry season compared to wet season.

Conclusion:
Soil samples from Kampung Orang Asli Sungai Lalang Baru, Ulu Semenyih is contaminated with *Ascaris lumbricoides*. This could play an important role in transmission of the STHs disease towards the villagers. For further study, it would be good to look into presence of parasite in the water samples and domestic animals surrounding the village, which could play an important role in STHs transmission.

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MORPHOLOGICAL CHARACTERISATION OF ISOLATED *Bacillus thuringiensis* AND SCREENING OF THE δ-ENDOTOXINS AND *chi* GENES.

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Background:
Microbial pesticides are naturally occurring biological agents that control target pests, yet remain safe for the environment. The most widely used microbial pesticides are from the strains of *Bacillus thuringiensis*. *Bacillus thuringiensis* is a Gram-positive, rod-shaped, facultative anaerobic and endospore-forming soil bacterium with the ability to produce parasporal crystals containing δendotoxins (Cry and Cyt toxins) that rendered it its insecticidal activity. Certain *Bacillus thuringiensis* strains were also shown to possess non-insecticidal crystal proteins, known as parasporin proteins that exhibit cytotoxicity, enabling them to kill human cancer cells.

Methods:
This study aimed to characterise the isolated *Bacillus thuringiensis* (A10C, D10D, N6BSS, 6A3, 8A3, 8A3S and 8B3) via morphological examination of the crystal proteins produced through scanning electron microscopy. Following that, screening of the isolates for *cry*, *cyt*, *chi* and *ps* genes were performed through PCR amplification.

Results:
Morphological examination of the bacterial isolates A10C, D10D, N6BSS, 6A3, 8A3, 8A3S and 8B3 confirmed the crystal protein produced were small, spherical in shape. The *cry1* gene was present in bacterial isolates N6BSS, 6A3, 8A3 and 8B3 while *cry2* gene was present in all of the bacterial isolates except 6A3 and 8B3. In addition, there were four isolates (A10C, N6BSS, 8A3 and 8B3) that showed positive results in *cry4* gene screening; and four isolates (A10C, N6BSS, 8A3 and 8A3S) that showed positive results in *cry11* gene screening. All bacterial isolates possessed *cry10* and *cyt2* genes. In *chi, ps1, ps2* and *ps4* gene screening, all of the bacterial isolates showed negative results.

Conclusion:
These results suggested that all of the *Bacillus thuringiensis* isolates proven with their insecticidal properties through the genetic characterisation, could be potentially utilised in controlling medical and agricultural pests. However, the absence of *ps* genes in these bacterial isolates indicated their inability to exhibit cytotoxicity towards cancer cells. Thus, it can be concluded that all the *Bacillus thuringiensis* isolates in this study are potential insecticidal strains.