DETECTION OF *TRYPANOSOMA* SPP. IN ASIAN SWAMP EEL, *MONOPTERUS ALBUS* IN SELECTED STATES IN MALAYSIA

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Detection of *Trypanosoma* spp. in Asian Swamp Eel, *Monopterus albus* in Selected States in Malaysia

By

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DETECTION OF TRYPANOSOMA SPP. IN ASIAN SWAMP EEL,

MONOPTERUS ALBUS IN SELECTED STATES IN MALAYSIA

ABSTRACT

An abstract of the research paper was presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement for the course DVT 55204 – Research Project.

Trypanosomiasis is one of the most important economic diseases affecting both marine and freshwater fish. Trypanosoma is a flagellated blood protozoan transmitted via leech parasitism in fish. Clinical signs in fish may manifest as emaciated, dullness, respiratory distress, mild ascites and anaemia. Asian Swamp Eels (*Monopterus albus*) are native to the tropical and subtropical regions in Asia and have the potential to become an economic aquatic commodities in Malaysia. This study aimed to detect the presence of *Trypanosoma* spp. in the swamp eel population across different locations in Kelantan, Terengganu and Perak. A total of 39 eels were collected from diverse locations each representing a diverse habitat of rivers, paddy fields and swamps. Detailed microscopic examination of the blood samples collected revealed no presence of trypanosomes. DNA extraction was done on the blood samples using the PrimeWay Genomic DNA Extraction Kit to obtain Trypanosoma genetic material. Kin1 and Kin2 primers were used in this study to detect the presence of *Trypanosoma* spp. The PCR analysis targeting specific Trypanosoma genes showed negative results for all the tested samples. As a result, no PCR products were forwarded for sequencing. The absence of trypanosomes detected may suggest a low risk of the diseases within these regions. However, it is essential to consider variations in environmental factors, habitats, and the dynamic nature of our aquatic ecosystems have influenced the study outcomes. Despite the negative results, the study contributes valuable insights into overcoming potential economic repercussions for Malaysia's aquaculture industry.

Keywords: Trypanosoma, Asian Swamp Eel, Malaysia, PCR Analysis

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Trypanosomiasis merupakan salah satu penyakit ekonomi yang penting yang memberi kesan kepada ikan marin dan air tawar. Trypanosoma adalah protozoa darah bersilia yang ditularkan melalui parasitisme lintah pada ikan. Tanda-tanda klinikal pada ikan mungkin termasuk kekurusan, kelesuan, kesukaran bernafas, sedikit asites dan anemia. Belut Sawah Asia (Monopterus albus) adalah asli kepada kawasan tropika dan subtropika di Asia dan mempunyai potensi untuk menjadi komoditi akuatik ekonomi di Malaysia. Kajian ini bertujuan untuk mengesan kehadiran *Trypanosoma* spp. dalam populasi belut sawah di lokasi yang berbeza di Kelantan, Terengganu dan Perak. Sejumlah 39 ekor belut telah dikumpul dari lokasi yang berbeza mewakili habitat yang beragam seperti sungai, sawah padi dan paya. Pemeriksaan mikroskopik terperinci terhadap sampel darah yang dikumpulkan tidak menunjukkan kehadiran tripanosom. Ekstraksi DNA dilakukan pada sampel darah menggunakan Kit Ekstraksi DNA Genomik PrimeWay untuk mendapatkan bahan genetik Trypanosoma. Primer Kin1 dan Kin2 digunakan dalam kajian ini untuk mengesan kehadiran *Trypanosoma* spp. Analisis PCR yang menargetkan gen Trypanosoma tertentu menunjukkan hasil negatif untuk semua sampel yang diuji. Sebagai hasilnya, tiada produk PCR yang dihantar untuk pensiriian. Ketidak hadiran tripanosom yang dikesan mungkin menunjukkan risiko rendah penyakit dalam kawasan ini. Walau bagaimanapun, adalah penting untuk mempertimbangkan variasi dalam faktor persekitaran, habitat, dan sifat dinamik ekosistem akuatik kita yang telah mempengaruhi hasil kajian. Walaupun hasilnya negatif, kajian ini memberikan wawasan berharga untuk mengatasi kesan ekonomi potensial terhadap industri akuakultur Malaysia.

Kata kunci: Trypanosoma, Asian Swam Eels, Malaysia, Analisis PCR

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Lab Assistants of FPV UMK

Family Members

DVM 5 Class of 2019/2024

Thank you.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
DNA	Deoxyribonucleic acid
PCR	Polymerase Chain Reaction
EDTA	Ethylenediaminetetraacetic acid

LIST OF SYMBOLS

%	Percentage

CHAPTER 1

INTRODUCTION

1.1 Introduction

Trypanosoma is a genus of kinetoplastids, from the phylum Sarcomastigophora which are protozoan parasites primarily restricted to invertebrate hosts causing trypanosomiasis. Trypanosoma is known to be pathogenic to humans and animals, thus transmitted through invertebrate vectors (Kaufer et al., 2017). Thus the transmission of Trypanosoma in fish occurs during leech parasitism (Martins et al., 2015). Trypanosoma is a parasitic organism that infects the circulatory system of vertebrates. It has a slender and flattened body shape, pleomorphic with a central nucleus. The flagellum emerges from a blepharoplast near the flagellated end, extending freely beyond the body. Reproduction occurs through binary or multiple fission (Gupta, 2006).

Trypanosomiasis is one of the most important economic diseases affecting freshwater fish. It had been reported to cause a major motility outbreak in Nile tilapia intensive farming in South America thus causing a substantial economic loss in the aquaculture industry (De Jesus et al., 2018). Trypanosomiasis in fish can be asymptomatic, however high numbers of parasitism in the blood circulation may severely affect the host and can cause anaemia, leukocytosis, hypoglycemia, splenomegaly (Islam & Woo, 1991) and thrombocytopenia (Fink et al., 2015) Therefore manifest to show clinical signs of emaciated, dullness, respiratory distress, loss of escape reflex, mild ascites, and paleness of the gills (Aly et al., 2005).

Monopterus albus or Asian Swamp eels belong to the order Synbranchiformes and the family of Synbranchidae which are known to be a group of eel-like percomorph fish

(Nico et al., 2019). Synbranchidae is considered as a fish-like eel or not a true eel as they lack pectoral and pelvic fins, compared to the other family Chaudhuriidae which have caudal fin which is not attached to the dorsal and anal fin, and Mastacembelidae, which has dorsal fin spines on the body (Fishes of the Cambodian Mekong, n.d.). *Monopterus albus* is commonly known by locals as the rice eel, rice field eel or rice paddy eel as it can be found in rice paddy fields. It is native to tropical and subtropical regions of India, China, Thailand, Philippines, Malaysia and Indonesia (Collins et al., 2002).

The natural habitat of these eels consists of stagnant waters (Shafland et al., 2009), shallow wetlands, ditches, ponds, streams, rivers, canals, lakes and reservoirs. These freshwater eels prey on small fish, worms, crustaceans and other small aquatic animals (Asian Swamp Eel (Monopterus Albus) - Species Profile, n.d.). Swamp eels thrive in muddy areas abundant with organic materials where the pH ranges from 3.6 to 6.5 thus these acidic environments are susceptible to opportunistic pathogens including the trypanosomes which is the most common occurrence in aquaculture.

1.2 Research problem

The detection of *Trypanosoma* spp. in Asian swamp eels (*Monopterus albus*) within the regions of Kelantan, Terengganu, and Perak is crucial due to the potential economic repercussions for Malaysia's aquaculture industry. Trypanosomiasis, caused by *Trypanosoma* spp. poses a significant threat, with the potential for substantial economic losses in the aquaculture sector. The burgeoning demand for freshwater eels in Malaysia underscores the importance of expanding eel aquaculture, making it a key player in the country's aquatic commodities. However, the risk of high mortality due to Trypanosoma infections presents a critical concern that necessitates a comprehensive study to understand the prevalence and impact of these parasites on Asian swamp eels. Despite the economic significance and potential for expansion, the

current understanding of Trypanosoma infections in freshwater fish, particularly Asian swamp eels, is inadequate. This lack of knowledge hinders effective management strategies and poses a potential threat to public health. The gaps in understanding the prevalence, distribution, and specific species of Trypanosoma in Asian swamp eels in the specified regions contribute to this knowledge deficit. In turn, this knowledge gap not only jeopardizes the health of the aquaculture industry but also poses a risk to public health, emphasizing the urgent need for a comprehensive investigation to fill these critical gaps in our understanding of Trypanosoma infections in Malaysian freshwater fish.

1.3 Research Questions

Are Asian Swamp Eels Kelantan, Terengganu and Perak infected with *Trypanosoma* spp.?

1.4 Research hypotheses

There is a presence of *Trypanosoma* spp. In the Asian Swamp Eel population in Kelantan, detected by cytology and molecular methods

1.5 Research Objectives

To detect the presence of *Trypanosoma* spp. In Asian Swamp Eels in Kelantan, Terengganu and Perak.

CHAPTER 2

LITERATURE REVIEW

2.1 *Trypanosoma* spp. infection in fish

High trypanosome prevalence (90-100%) and parasitemia have been reported based on a study conducted on Brazilian armoured catfish and leeches (Lemos et al., 2015). A recent study reported that the prevalence of Trypanosoma is at 9.76% and 41.32% respectively in the west and east of peninsular Malaysia (Hassan et al., 2012). Histopathological lesions of the protozoan infection can be observed in the gills, liver, pancreas, spleen, kidney and heart (De Jesus et al., 2018). The protozoan can be identified from histopathology analysis and for instinct it can be seen within the blood vessel and multiple pigmented areas of melanomacrophage aggregation in the spleen (De Jesus et al., 2018). Movement of the Trypanosoma in the blood circulation can induce mechanical injury towards the RBC as the parasite has a spiral waveform and rotates like a corkscrew-like motion with a high velocity (Safar & Azab, 2009).

2.2 Presence of *Trypanosoma* spp. in fish

Fish is an important protein source for people in Asia. In Vietnam, Asian Swamp Eels have been cultured (Sieu et al., 2009) and are considered a popular delicacy in Asian countries as they are farmed in polyculture rice fields and sold as food products. Preliminary studies of a parasitic infection of freshwater eels in Malaysia also found that swamp eels in Malaysia tend to host Trypanosoma, Cestoda, Digenia and Nematoda (Hassan et al., 2012).

According to Lemos et al. (2015), more than 60 *Trypanosoma* spp. have been documented in both marine and freshwater fishes in Brazil. Notable among thesis include *Trypanosoma hypostomi*, *Trypanosoma chagasi*, *Trypanosoma guaibensis* and *Trypansooma lopesi*. Moreover, a new species, *Trypanosoma abeli* was newly identified, recorded and characterized phylogenetically which showed a high prevalence in armoured catfish species in South America (Lemos et al., 2015).

2.3 Detection of *Trypanosoma* spp.

Trypanosoma can be detected by microscopic examination by using Giemsa-stained blood films. Examination through direct wet mount using blood collected without anticoagulant can demonstrate the motility of the parasite. Trypanosomes are often present in blood samples at low intensities therefore to increase the chances of detecting the protozoan, Gupta (2006) suggested techniques of the haematocrit centrifuge method (Woo,1969), and Clot method (Lom et al 1992). Both of these methods demonstrate observing the flagella movement of the Trypanosoma from the buffy coat and the blood serum. The presence of the protozoan can also be detected through histopathological observations thus present within blood vessels and can be found in various organs (De Jesus et al., 2018). Molecular methods have been shown to detect and identify the presence of *Trypanosoma* spp., using Polymerase Chain Reaction (PCR) thus enabling us to detect the parasite genetic material even at low concentrations of parasitism. (Lemos et al., 2015)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Sampling

A total of 39 Asian Swamp Eels (*Monopterus albus*) were collected from three different locations, namely Kelantan, Terengganu, and Perak. The swamp eels were transported alive in aerated plastic bags to the laboratory. Upon arrival at the laboratory, the eels were transferred into the aerated aquarium and acclimatized overnight before blood sampling was done.

3.2 Fish anaesthetic

The eels were anaesthetized in an ice bath in the laboratory for 5 minutes. Within 5 minutes, causing the muscles to be relaxed. Body weight and total body length were measured using a weighing scale and ruler.

3.3 Blood collection

Blood was withdrawn by puncturing the caudal vessels using a disposable 3 ml syringe and 21G needle. Blood was immediately transferred into Ethylene Diamine Tetraacetic Acid (EDTA) blood collection tubes. Fresh blood was also immediately processed for wet mount and thin blood smear.

3.4 Blood smear

Fresh blood was immediately processed for wet mount and thin blood smear. A wet mount slide was done to detect the presence of *Trypanosoma* spp. The slide was then immediately observed under a compound microscope. Thin blood smears were prepared

on glass slides and air-dried. Slides were fixed in pure methanol for 1 minute and stained with Giemsa solution for 45 minutes, rinsed with tap water and air dried before observed. Four thin blood smears were prepared for each blood sample.

3.5 DNA extraction

DNA extraction was done using the PrimeWay Genomic DNA Extraction Kit (PrimWay, US). Blood samples from EDTA tubes at room temperature were used for this process, and the steps were as follows, according to the DNA extraction protocol. First, 200 µL of the sample was transferred into a new 1.5 ml microcentrifuge tube. For the lysis of the blood sample, 25 µL Proteinase K was added to the samples. 200 µL TBL2 Buffer was added and vortexed vigorously for 10-20 seconds to mix. The sample was then incubated at room temperature for 5 minutes and mixed by vortexing. Then, the sample was incubated at 70°C for 10-15 minutes. For the binding process, 210 µL ethanol was added to the sample and mixed by vortexing. Then, a PrimeWay Genomic Column was placed into a collection tube. The sample was transferred into the PrimeWay Genomic Column and centrifuged at 11,000 x g for 1 minute. Then the collection tube was discarded. The PrimeWay Genomic Column was then placed into a new collection tube.

Continuing with the washing process, 500 µL Wash Buffer T1 was added to the column and centrifuged at 11,000 x g for 1 minute. The flow-through was discarded, and the column was placed back into the collection tube. Then, 600 µL Wash Buffer T2 was added to the column and centrifuged at 11,000 x g for 1 minute. The flow-through was discarded, and the column was placed back into the collection tube. For drying, the sample was then centrifuged again at 11,000 x g for 1 minute to remove the ethanol residue. Lastly, for the elution of the DNA sample, the PrimeWay Genomic Column was placed into a new 1.5 ml centrifuge tube. A 100 µL Elution Buffer was added at the centre of the

column membrane and left to stand at room temperature for 1 minute. The PrimeWay Genomic Column was then centrifuged at 11,000 x g for 1 minute to elute the DNA.

3.6 Polymerase chain reaction

Kin1 and Kin2 primers were used in this study to detect the presence of Trypanosoma spp. Based on a previous study conducted by Desquesnes et al. (2001). Primer sequences: Kin1, 5'GCG TTC AAA GAT TGG GCA AT-3' (reverse); Kin2, 5'-CGC CCG AAA GTT CAC C-3' (forward). The reagent used and the volume for a single PCR reaction using a T100 Thermocycler (Bio-Rad, USA) was a master mix (PCR buffer, Mg+, Cl-, DNTP, Taq polymerase) with a volume of 12.5 µl, forward primer 1µl, reverse primer µl, nuclease-free water 8.5µl and a sample of 5 µl. PCR protocol used was as follows: an initial step of 3 min at 94℃; four cycles of amplification with 1 min denaturation at 94℃, 1 min hybridization at 58℃ and 1 min elongation steps at 72℃; eight cycles of amplification with 1 min denaturation at 94℃, 1 min hybridization at 56℃ and 1 min elongation steps at 72℃; 23 cycles of amplification with 1 min denaturation at 94℃, 1 min hybridization at 54℃ and 1 min elongation steps at 72℃; and a final extension step at 5 min at 72℃.

3.7 Agarose gel electrophoresis

Amplified PCR products were visualised on a 1.5% agarose gel and stained with Midori green. The electrophoresis set was run at 100V and 400mA for 40 mins. The electrophoresis gel was then photographed using a gel documented system, UV Transilluminator (Bio-Rad USA)

CHAPTER 4

RESULTS

4.1 Sample demographic

A total of 39 Asian Swamp eels were obtained from three different locations within Kelantan, Terengganu and Perak. The eels were collected from diverse locations each representing distinct habitats. The collection sites encompassed rivers, paddy fields and swamps respectively.

Table 4.1 Sample demographic of Asian Swamp Eel

Location	Habitat	Number of eel	Mean body length (cm)	Mean body weight (g)
Kelantan	River	12	50.41	65.42
Terengganu	Paddy field	22	44.80	56.69
Perak	Swamp	5	65.09	312.84

4.2 Microscopic examination

Detailed microscopic examinations of the collected samples revealed no presence of *Trypanosoma* spp. in any of the specimens from Kelantan, Terengganu and Perak.

4.3 Molecular analysis

Utilizing molecular techniques including PCR showed no evidence of Trypanosoma spp. DNA in any of the samples of Asian Swamp eels. The PCR analysis targeting specific Trypanosoma genes showed negative results for all the tested samples. As a result, no PCR products were forwarded for sequencing. (Appendices)

CHAPTER 5

DISCUSSION

The absence of *Trypanosoma* spp. detected in the Asian Swamp eel population may suggest a low risk of trypanosomiasis in these regions. However, several key factors need to be considered and therefore may have influenced the outcome of the study. Previous studies show a relatively high prevalence of trypanosomes and other parasites in Asian Swamp eels (Ihwan et al., 2013, Mahasri et al., 2019) and other freshwater fish in Malaysia (Hassan et al., 2012). Therefore, environmental factors, habitat and timeline of the study differ from the initial studies reported.

The method of detection used may have influenced the outcome of the study. The absence of the trypanosome within the microscopic examination may also be due to the low parasite load within the organism. Microscopic examination is considered the golden standard for trypanosome detection. Common techniques include examination of buffy coat, wet blood mount, thin and thick blood smears followed by Giemsa staining. Microscopic examination is the most effective diagnostic approach due to its simplicity, cost-effectiveness, and ability to identify another blood parasite simultaneously (Katsidzira & Fana, 2010). Despite its high specificity, it has low sensitivity, especially in detecting early infection or low parasitaemia (Odiit et al., 2005).

In comparison, molecular techniques such as polymerase chain reaction (PCR) have a significantly high level of sensitivity and accuracy in the detection of blood parasites and can be applied in the detection of *Trypanosoma* spp. (Thumbi et al., 2008). However, the method requires resources, facilities, high cost and in need of trained personnel. Molecular tests can detect and differentiate the trypanosome species, however,

they require specific primers to amplify the target DNA regions (Ferreira et al., 2014). Therefore, PCR methods are highly likely to have experimental errors and contamination (Pienaar et al., 2006).

Trypanosoma spp. is known to be transmitted to fish and other aquatic animals via leeches and is considered the main vector of this disease's transmission (Lemos et al., 2015). Leeches are present in ecosystems ranging from terrestrial and freshwater to estuarine and marine environments. These annelids breed in mud ponds, swamps and rice fields thus sharing the same habitat as the Asian swamp eels (Phillips et al., 2020).

The presence of pollutants and chemicals from herbicides and pesticides within the environment may pose a threat towards the population of leeches within the ecosystem. According to a recent study conducted by Zhou (2023), these environments contain all sorts of pollution including pesticides and herbicides hence exposure to these chemicals over a long period may have an effect towards their life cycle. Hence, 26 types of pesticide residues were able to be detected in the leeches. Moreover, pollutants within the environment may affect the reproductive cycle of the leech. The eggs are more likely to be harmed by the harsh chemicals in the water as they lack a protective shell and are instead enclosed in a gelatinous mass or capsule (Watts, 2023). Therefore, pesticides and herbicides within the water especially in paddy fields may have caused a decline population of leeches thus reducing the risk of these vector-borne diseases such as trypanosomes in aquatic animals.

According to a recent study by Phillips et al. (2020), leeches are hardy and hence very well adaptable to environmental changes and exposure to extreme pH and pollution. Leeches are versatile creatures found in diverse and challenging environments characterized by extremes in temperature, moisture, salinity, pressure, light, and pollution. Whereas some leech species exhibit specialized adaptations in morphology, physiology, or behaviour to thrive in extreme conditions. Interestingly, leeches adapted

to inhospitable habitats are distributed across various branches or families in leech phylogeny, indicating multiple independent invasions into environments with extreme conditions.

Prolonged exposure to the pollutants and pesticide residue that resides within the leech may have a negative effect towards the lifecycle of the trypanosome. There is no reproduction of trypanosomes in fish therefore, they rely on leeches as intermediate hosts. The life cycle of trypanosomes initiates in leech digestive tracts as sphaeromastigotes, transforming into infective metatrypansomes in the leech's proboscis. These metatrypansomes are transmitted to fish during leech feeding. In fish, development spans 42-62 days, with small trypomastigotes appearing in the blood at day 3 post-infection. They mature into mastigotes within 29-55 days, and as parasitemia decreases, pleomorphic trypomastigotes become more prominent—trypomastigotes in fish blood exhibit size variations based on the stage of infection (Chong, 2005).

The absence of trypanosomes may simply reflect the ecosystem's health. According to Lymbery et al. (2010), endoparasites with intricate life cycles are particularly vulnerable to the adverse effects of environmental changes. This vulnerability arises from the idea that any modifications in the environment affecting either the free-living stages or any of the hosts engaged in parasite transmission could diminish parasite population size, potentially resulting in local extinction. Therefore the prevalence of parasites in aquatic species offers valuable insight into the biological availability of pollutants, the contaminant effects of pollutants on the free-living stages of parasites and the ecosystem health (Sures et al., 2017).

CHAPTER 6

CONCLUSION

The comprehensive investigation into the presence of *Trypanosoma* spp. in Asian Swamp eels (*Monopterus albus*) within Kelantan, Terengganu, and Perak revealed no evidence of infection through both detailed microscopic examinations and PCR. The absence of *Trypanosoma* spp. in the collected samples suggests a potential low risk of trypanosomiasis in these regions. However, it is essential to consider the detection method used, environmental factors, and the influence of pollutants on the lifecycle of intermediate hosts, leeches and trypanosomes. Despite the negative results, the study contributes valuable insights into the health status of Asian Swamp eels and the ecosystem in the specified regions.

CHAPTER 7

RECOMMENDATION

Several recommendations emerge to guide future research and management efforts to further study the absence of Trypanosoma spp. in the Asian Swamp eels (Monopterus albus) studied in Kelantan, Terengganu, and Perak,

Firstly, future studies should implement a combination of microscopic examination and molecular techniques as for comprehensive surveillance of the Trypanosoma spp., in Asian Swamp eels. Due advancement of future technology, I would recommend the usage of other available primers that may have a higher specificity towards the specific Trypanosoma spp infecting the aquatic organism. This dual approach can provide a more accurate assessment of infection prevalence ensuring both sensitivity and specificity in detection.

Considering the dynamic nature of aquatic ecosystems, it is advisable to initiate continuous monitoring programs. Regular surveillance would contribute to the ongoing assessment of the prevalence and distribution of trypanosome infection in Asian Swamp eels, helping to identify potential changes over time and variations across different locations.

Understanding the environmental factors influencing trypanosome infection is essential therefore future research should delve into the relationship between water quality, the presence of intermediate hosts, trypanosome and ecosystem. Particular focus on the focus on pollutants and pesticides may influence the population's health. Such investigations would provide valuable insights into the ecological conditions that favour or deter the presence of trypanosomes in Asian Swamp eels.

Furthermore, long-term ecological studies should be initiated to monitor the changes in the prevalence of Trypanosoma spp. and other parasites in Asian Swamp eels. Such studies should also consider the influences of seasonal variation, climatic factors, and anthropogenic activities on the overall health of aquatic ecosystems. Moreover with the given potential role of leeches as vectors for Trypanosoma spp. studies should assess the leech population, abundance, distribution and potential effects when exposed to herbicides and pesticides. Understanding the dynamics of these elements can contribute to insights into the transmission of trypanosome infections.

Public awareness campaigns targeting fish farmers and local communities should be initiated to educate the public on the significance of biosecurity and measures in aquaculture. Promoting the best practices can contribute to preventing the introduction and spread of parasitic infection and safeguarding the health of aquatic ecosystems and aquaculture industry.

Lastly, while this study focused on *Trypanosoma* spp. I recommend collaborative research efforts among researchers, environmental scientists and many more to tackle the complex issue related to trypanosome transmission. This can facilitate the exchange of expertise and resources leading to more effective strategies for disease management and prevention in our aquaculture industry.

APPENDIX

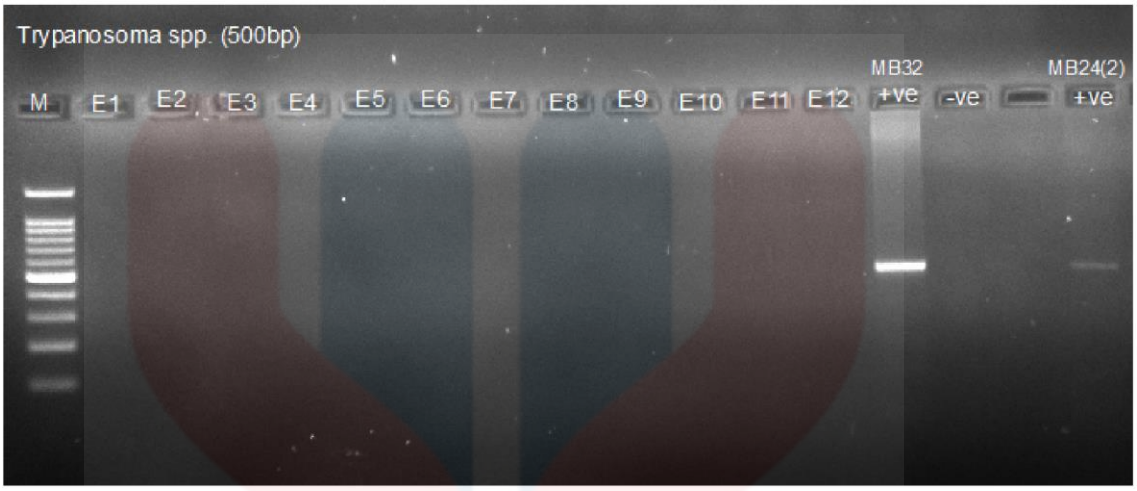

Figure 1: Gel electrophoresis of samples from Kelantan.

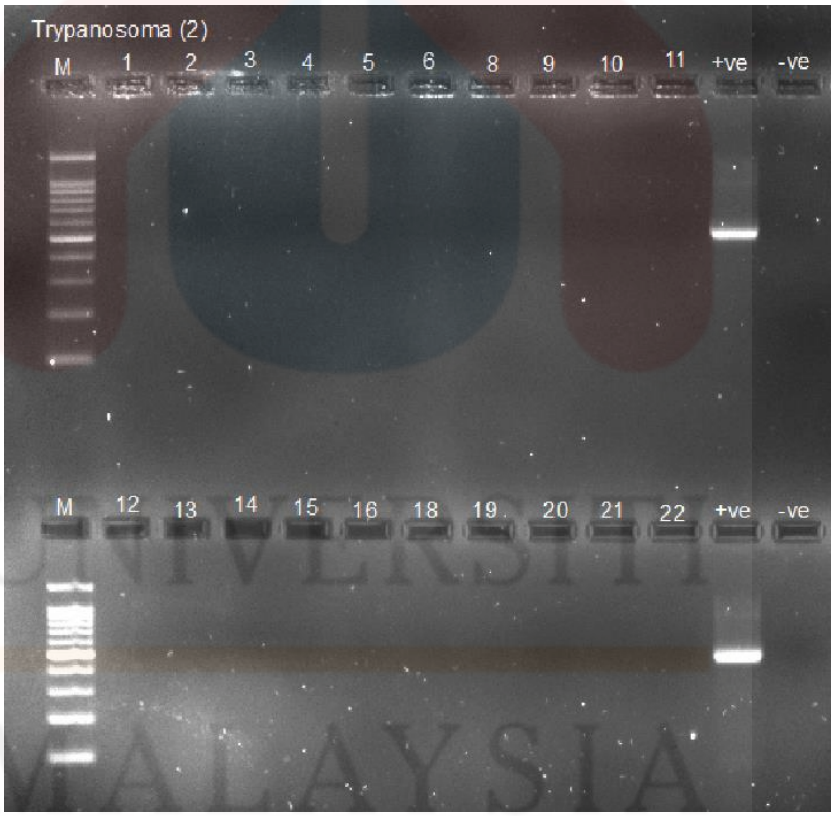

Figure 2. Gel electrophoresis of samples from Terengganu.

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