UNIVERSITI PUTRA MALAYSIA

SCREENING FOR PISCINOODINIASIS, ICHTHYOPHTHIRIASIS, MYCOBACTERIOSIS AND EPIZOOTIC ULCERATIVE SYNDROME IN TWO WILD POPULATIONS OF PEACEFUL BETTA (*Betta imbellis)* IN SELANGOR

ANDI ALTAFF BIN ASMAR

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FACULTY OF VETERINARY MEDICINE
UNIVERSITI PUTRA MALAYSIA
SERDANG, SELANGOR

2023/2024

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ANDI ALTAFF BIN ASMAR

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
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CERTIFICATION

It is hereby certified that I have read this project paper entitled "Screening For Piscinoodiniasis, Ichthyophthiriasis, Mycobacteriosis And Epizootic Ulcerative Syndrome In Two Wild Populations Of Wild Peaceful Betta (*Betta imbellis*) In Selangor", by Andi Altaff bin Asmar, and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

ASSOC. PROF. DR MD SABRI MOHD YUSOFF
DVM, MSc, PHD (UPM),
Faculty of Veterinary Medicine,
University Putra Malaysia
(Supervisor)

DOCTOR MOHD FUAD BIN MATORI
DVM (UPM),
Faculty of Veterinary Medicine,
University Putra Malaysia
(Co-Supervisor)

DEDICATION

This project paper is a tribute to Allah, the Creator who has not only shaped my existence but has also been the guiding force behind every aspect of this project.

To my family,

My mother, Mailizah bte Hafezah,

My father, Asmar bin Andi Yakin,

My maternal grandmother, Maimunah binti Maidu,

My paternal grandmother, Aminah binti Mangussara

My friends,

I want to express my heartfelt gratitude to all my mentors and teachers who have passionately devoted themselves to the noble cause of education. Your unwavering support and caring guidance mean the world to me.

May this stand as a wellspring of inspiration and motivation for your forthcoming endeavours.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
EUS	Epizootic ulcerative syndrome
spp	Species
±	Plus minus
x	Times
g	Gram
ml	Millilitre
RM	Ringgit Malaysia
pH	Potential of hydrogen

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian keperluan kursus VPD4999 – Projek Tahun Akhir

Pemeriksaan bagi Piscinoodiniasis, Ikhtiophtiriasis, Mikobakteriosis, dan Sindrom Ulseratif Epizootik dalam Dua Populasi Liar Ikan Laga Kampung (*Betta imbellis*) di Selangor.

Oleh

Andi Altaff bin Asmar

2023

Penyelia: Prof. Madya Dr Md Sabri Mohd Yusof

Penyelia bersama: Prof. Dr Mohd Fuad Matori

ABSTRAK

Peningkatan popularitи global ikan laga kampung (*Betta imbellis*) dalam industri ikan hiasan menimbulkan kebimbangan, terutama memandangkan bekalan ikan induk sering berasal dari populasi liar. Kehadiran penyakit dalam ikan liar membawa ancaman yang signifikan kepada populasi yang dipelihara. Walaupun penyelidikan yang meluas telah dijalankan pada *Betta splendens*, menjelajahi kerentanan mereka terhadap penyakit seperti piscinoodiniasis, ikhtiophtiriasis, mikobakteriosis, dan sindrom ulseratif epizootik, penyelidikan serupa mengenai kerentanan *B. imbellis* kurang. Kajian ini bertujuan untuk menangani kesenjangan pengetahuan ini dengan menilai kekerapan berlakunya penyakit dalam dua populasi *B. imbellis* liar di Klang dan menganalisis perbezaan prevalens penyakit di antara mereka. Dua puluh spesimen *B. imbellis* ditangkap pada dua lokasi dan ujian kualiti air dijalankan serentak. Ikan yang ditangkap mengalami euthanasia, nekropsi, dan pemeriksaan

menyeluruh, termasuk analisis makroskopik dan mikroskopik organ-organ berbeza (kulit, insang, hati, buah pinggang, dan tisu otot) untuk mengesan lesi dan patogen. Keputusan menunjukkan bahawa semua spesimen yang diperiksa sepenuhnya bebas dari keempat-empat penyakit yang diperiksa. Selanjutnya, tiada perbezaan prevalens penyakit antara dua populasi tersebut. Secara ringkasnya, kajian ini mendedahkan ketiadaan yang luar biasa bagi keempat-empat penyakit dalam *B. imbellis* liar, mungkin boleh dikaitkan dengan faktor-faktor seperti saiz sampel yang terhad, pemangsaan ikan yang dijangkiti, kematian semulajadi, atau keadaan yang tidak menyenangkan bagi patogen. Walau bagaimanapun, keaslian kelangkaan penyakit-penyakit ini dalam *B. imbellis* liar masih tidak pasti. Ini menekankan keperluan untuk penyelidikan yang lebih komprehensif, dengan saiz sampel yang lebih besar, untuk mendapatkan pandangan mendalam mengenai status kesihatan *B. imbellis* liar, dan dengan itu memberikan sumbangan yang signifikan kepada perbincangan saintifik yang berterusan dalam bidang ini.

Kata kunci: *Betta imbellis, Mikobakteriosis, Sindrom ulseratif epizootik, Ikhtiophtiriasis, Piscinoodiniasis.*

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD4999 – Final Year Project

Screening For Piscinoodiniasis, Ichthyophthiriasis, Mycobacteriosis And Epizootic Ulcerative Syndrome In Two Wild Populations Of Wild Peaceful Betta (*Betta imbellis*) In Selangor

By

Andi Altaff bin Asmar

2023

Supervisor: Assoc. Prof. Dr Md Sabri Mohd Yusof

Co-Supervisor: Dr. Mohd Fuad Matori

The increasing global popularity of peaceful bettas (*Betta imbellis)* in the ornamental fish trade has raised concerns, particularly considering that breeding stocks are often derived from wild populations. The presence of diseases in wild fish poses a significant threat to captive populations. While extensive research has been conducted on domesticated *Betta splendens*, exploring their susceptibility to diseases such as piscinoodiniasis, ichthyophthiriasis, mycobacteriosis, and epizootic ulcerative syndrome, similar investigations on the susceptibility of *B. imbellis* is lacking. This study aimed to address this knowledge gap by assessing the frequency of disease occurrence in two wild *B. imbellis* populations in Klang and analysing the disparities in disease prevalence between them. Twenty *B. imbellis* specimens were

netted at two sites and water quality tests were conducted simultaneously. The captured fish were subjected to euthanasia, necropsy, and thorough examination, including macroscopic and microscopic analyses of various organs (skin, gills, liver, kidney, and muscle tissue) to detect lesions and pathogens. The results showed that all specimens examined were completely free of the four diseases examined. Furthermore, there was no difference in disease prevalence between the two populations. In conclusion, the research showed that wild B exhibited an astonishing lack of all four diseases. imbellis, possibly caused by things like small sample sizes, fish that are already infected being eaten, natural mortality, or unfavourable environmental conditions for pathogens. But the veracity of these illnesses' rarity in wild B. Imbellis is still up in the air. This emphasizes how important it is to conduct more thorough studies with bigger sample sizes in order to gain a thorough understanding of the health condition of wild B. imbellis, and so add a great deal to the current scientific conversation in this area.

Keywords: *Betta imbellis, mycobacteriosis, epizootic ulcerative syndrome, ichthyophthiriasis, piscinoodiniasis.*

1.0 INTRODUCTION

1.1 Background

Betta imbellis (Ladiger, 1975), also known as Peaceful Betta, is an anabantoid species inhabiting sluggish water bodies in southern Thailand, Malaysia and Indonesia (Panijpan et al, 2020). Like other anabantoid species, *B. imbellis* possess suprabranchial chambers that house the labyrinth apparatus. This apparatus allows *B. imbellis* to extract atmospheric oxygen from the air. Their adaptability and ease of husbandry make *B. imbellis* a suitable species to keep as an ornamental fish. The local price of a male *B. imbellis* is approximately RM50 with competition grade males ranging from RM120-150.

In recent years, the surge in popularity of *B. imbellis* as an ornamental fish has led to an increased demand for breeding, resulting in the harvesting of wild populations. This practice, however, raises concerns as it exposes captive *B. imbellis* to potential diseases transmitted by their wild counterparts. While there's limited research on the health status of wild *B. imbellis*, studies on a closely related species, *Betta splendens*, indicate their susceptibility to piscinoodiniasis, fin rot, and mycobacteriosis (Lichak et al., 2022). Additionally, bettas are prone to ichtyophthiriasis, posing a risk of contagion to other fish.

Of particular concern is mycobacteriosis, caused by *Mycobacterium* spp., which is not only a threat to fish but also a potential public health risk. This zoonotic disease has been known to infect handlers of diseased fish (Decostere et al., 2004), manifesting in humans as "fish tank granuloma" or "fish handler’s disease," leading to granulomatous skin lesions.

Another alarming disease is epizootic ulcerative syndrome (EUS), caused by *Aphanomyces* sp. EUS first emerged in North Malaysian rice-crop fish in 1980 and has since been a major concern for both freshwater and brackish water fish (Kumar et al., 2020). It is crucial to note that EUS is recognized as an OIE notifiable disease.

Given these considerations, this study aims to investigate the prevalence of these diseases in *B. imbellis*. By doing so, it seeks to enhance our understanding of the health dynamics within wild populations, shedding light on critical aspects of this relatively understudied species. The findings are anticipated to contribute valuable information to the ongoing discourse on the health and conservation of *B. imbellis* in both wild and captive settings.

Justification

There is little research on the health status of wild *Betta imbellis* in Malaysia. *B. imbellis* has great potential in the ornamental fish industry. However, a lack of understanding of the health status of *B. imbellis* may result in the disease spreading to captive breeding populations. Furthermore, bettas are known to transmit zoonotic diseases of public health concern. Some disease that has not been seen in Malaysia for years may have the potential to re-emerging.

Studying the health of wild *B. imbellis* can improve understanding of diseases transmitted by bettas, take preventive measures to avoid infection of captive populations, and raise awareness of possible zoonotic and re-emerging diseases.

Objective of study

To investigate the occurrence of piscinoodiniasis, ichthyophthiriasis, mycobacteriosis, and epizootic ulcerative syndrome, and to compare the occurrence of these diseases between two wild populations of *B. imbellis* in Selangor.

Hypothesis of study

H_{01}: There will be no occurrence of piscinoodiniasis, ichthyophthiriasis, mycobacteriosis, and epizootic ulcerative syndrome in wild *B. imbellis* in Selangor.

H_{a1}: There will be occurrence of piscinoodiniasis, ichthyophthiriasis, mycobacteriosis, and epizootic ulcerative syndrome in wild *B. imbellis* in Selangor.

H_{02}: There will be no difference in occurrence of piscinoodiniasis, ichthyophthiriasis, mycobacteriosis, and epizootic ulcerative syndrome between two wild populations of *B. imbellis* in Selangor.

H_{a2}: There will be a difference in occurrence of piscinoodiniasis, ichthyophthiriasis, mycobacteriosis, and epizootic ulcerative syndrome between two wild populations of *B. imbellis* in Selangor.

2.0 Literature review

2.1 Distribution of wild *Betta imbellis*

Betta imbellis, commonly known as the Peaceful Betta, is a fascinating anabantoid species identified by Ladiger in 1975. This captivating fish is native to the calm waters of southern Thailand, Malaysia, and Indonesia, as noted by Panijpan et al. in 2020. Much like other anabantoids, *Betta imbellis* possesses unique suprabranchial chambers housing the labyrinth apparatus, a feature allowing them to extract atmospheric oxygen from the air. This remarkable adaptation makes them well-suited for living in slow-moving water bodies.

The peaceful Betta's versatility and straightforward care requirements make it an ideal choice for ornamental fish enthusiasts. Their ability to thrive in captivity adds to their charm, making them a popular choice among hobbyists. In the local market, a male *Betta imbellis* typically fetches around RM50, while those with competition-grade attributes can range from RM120 to RM150. This not only reflects their aesthetic appeal but also emphasizes their value within the ornamental fish community.

Figure 1: Distribution of selected nest-building Betta species in Thailand and neighbouring countries. (Panijpan et al., 2020)

2.2 Market value as ornamental fish

Recent findings from the Department of Fisheries underscore Malaysia's ascendancy as the eighth-largest global producer of ornamental fish, with a substantial majority (over 70%) destined for international markets. This study delves into the diverse taxonomy of ornamental fish cultivated in Malaysia, encompassing various freshwater species across families such as Anabantidae, Callichthyidae, Characidae,

Cichlidae, Cyprinidae, Cyprinodontidae, Loricariidae, Osteoglossidae, and Poecilidae (Department of Fisheries Malaysia, 2019).

Notably, the Anabantidae family, including *Betta imbellis*, emerges as a significant contributor, accounting for approximately RM22,000 in the overall ornamental fish export value (see Figure 2). Malaysia's standing as the second-largest contributor to global trade, constituting a noteworthy 9% share, underscores its strategic importance (Othman et al., 2017). *B. imbellis*, in particular, assumes a pivotal role in Malaysia's ornamental fish trade among diverse freshwater species.

On the international stage, Malaysia reaps substantial economic benefits, recording an income of $1,133 million from ornamental fish exports to European Union countries, as reported by the European Ornamental Fish Import and Exports. Key players in this global trade include Hong Kong, Japan, and Taiwan. While the burgeoning ornamental fish industry has brought economic prosperity, it concurrently poses challenges, with disease outbreaks standing out prominently.

Over the past two decades, disease outbreaks have become a pervasive issue for aquaculture farms, resulting in considerable economic losses. Predominantly of bacterial and viral origin, these diseases pose substantial threats to the health of ornamental fish. The emergence of global diseases further compounds challenges faced by aquaculture farms. Addressing these disease-related issues is imperative for sustaining the growth of Malaysia's ornamental fish industry.

PENGELUARAN DAN NILAI IKAN HIASAN, 2019
Production and Value of Ornamental Fish, 2019

IKAN HIASAN *Ornamental Fish*	2018		2019		PERUBAHAN / *Changes (%)*	
	Kuantiti (Ekor) *Quantity (Pieces)*	Nilai *Value* (RM '000)	Kuantiti (Ekor) *Quantity (Pieces)*	Nilai *Value* (RM '000)	Kuantiti *Quantity*	Nilai *Value*
Cyprinids	100,103,414	112,097.51	99,653,669	98,058.43	-0.45	-12.52
Anabantids	19,694,038	24,137.80	22,645,770	39,818.66	14.99	64.96
Poecilids	45,243,216	13,047.37	44,601,416	22,048.18	-1.42	68.99
Characins	27,595,078	23,865.73	21,515,668	20,114.76	-22.03	-15.72
Chichlids	9,191,730	13,358.60	11,091,199	138,786.31	20.66	938.93
Osteoglossids	765,873	107,794.34	1,046,800	139,370.32	36.68	29.29
Callichtyids	46,860,027	44,474.38	200	1.50	-100.00	-100.00
Cobitids	-	-	-	-	-	-
Loricariidae	13,087,508	3,037.02	6,160,689	1,233.08	-52.93	-59.40
Lain-lain *(Others)*	62,787,619	8,513.39	80,816,128	47,016.56	28.71	452.27
JUMLAH BESAR *Grand Total*	**325,328,503**	**350,326.14**	**287,531,539**	**506,447.79**	**-11.62**	**44.56**

Figure 2: Production and value of ornamental fish in Malaysia (Department of Fisheries Malaysia, 2019).

2.3 Piscinoodiniasis

The present study highlights the significance of 'velvet' disease, also known as pscinoodiniasis in *Betta* spp., with specific emphasis on its aetiology, pathogenesis, and preventive measures. Goldstein (2004) identifies this condition as a pertinent concern, attributing its causation to the dinoflagellate *Piscinoodinium* spp. The vulnerability of young fry to infection and the subsequent development of clinical manifestations are notably pronounced, rendering effective preventive strategies paramount.

Piscinoodinium spp. thrives abundantly in water characterized by suboptimal quality. The manifestation of infection primarily affects the skin and gills, inducing hyperplasia, haemorrhage, compromised osmoregulation, and necrosis, as

expounded upon by Roberts et al. in 2009. The developmental stage of fry amplifies their susceptibility to the disease, necessitating a comprehensive understanding of its pathogenesis for effective preventive measures.

Prevention assumes pivotal importance in managing 'velvet' disease, with Goldstein (2004) underscoring that fry, once exhibiting clinical signs, often succumb to the ailment. This necessitates proactive measures to curtail the prevalence of the dinoflagellate and mitigate conditions conducive to its flourishing. A comprehensive exploration of the etiological factors, pathological processes, and preventive strategies is imperative for a nuanced understanding of 'velvet' disease in *Betta* spp.

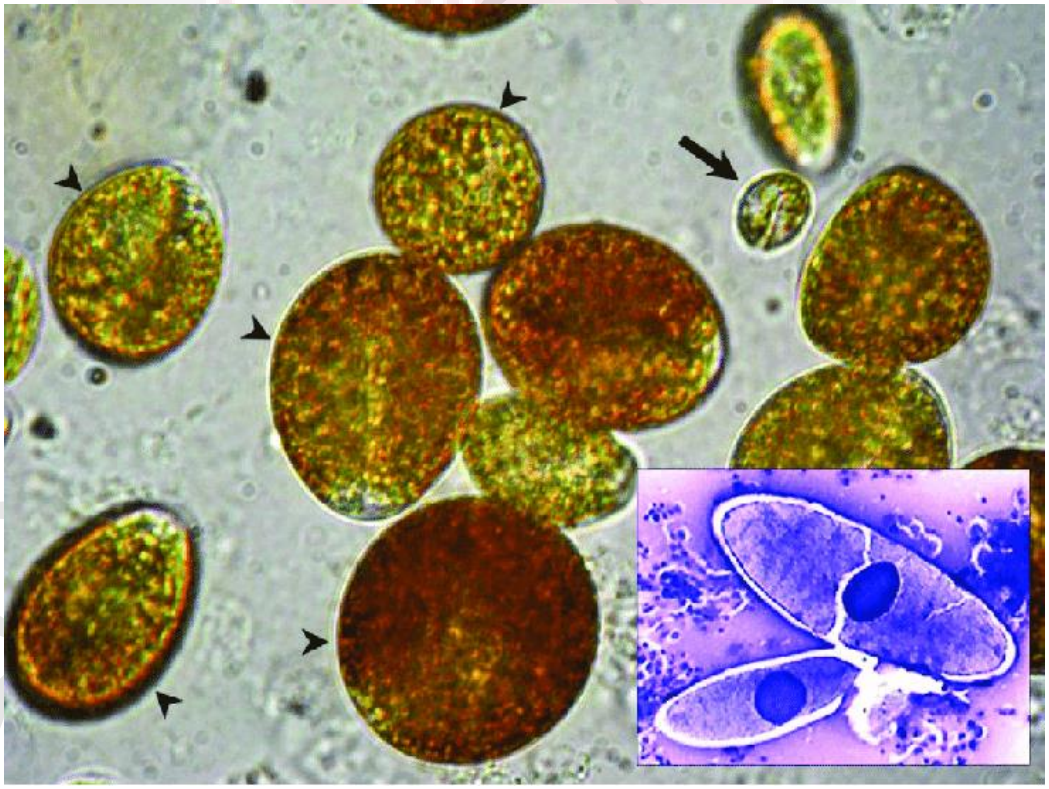

Figure 3: Piscinoodinium spp. *(Martins et al., 2015).*

2.4 Ichthyophthiriasis

White spot disease, scientifically recognized as *Ichthyophthirius multifilis*, stands as the prevailing protozoan affliction in freshwater fish. Characterized by a direct lifecycle devoid of intermediate hosts, this ciliate protozoan progresses through distinct stages - trophont, tomite, and theronts. The trophont, infiltrating and encysting between the skin and gill epithelium of the fish host, manifests as distinct white blisters, a hallmark of the malady (Buchmann, 2019). Under 40x magnification, identification of *Ichthyophthirius multifilis* is facilitated by the observation of a sizable, oval structure housing a horseshoe-shaped macronucleus, as depicted in Figure 4 (Dickerson, 2012).

Widespread in its distribution, this pathogenic protozoan populates diverse regions, ranging from tropical and subtropical to temperate zones, extending northward to the Arctic Circle (Matthews, 1994; Elsayed et al., 2006). *Ichthyophthirius multifilis* precipitates severe epizootics within aquaria, hatcheries, and ponds, exerting its impact not only on captive populations but also in the natural habitats of various fish species (Ezz El-Dien et al., 1998; Kim et al., 2002; Thilakaratne et al., 2003).

This comprehensive examination illuminates the intricate lifecycle, clinical manifestations, and widespread distribution of *Ichthyophthirius multifilis*. Acknowledging its pervasive impact across diverse environments underscores the imperative for continued research and strategic measures to manage and mitigate the detrimental effects of this common freshwater protozoan pathogen.

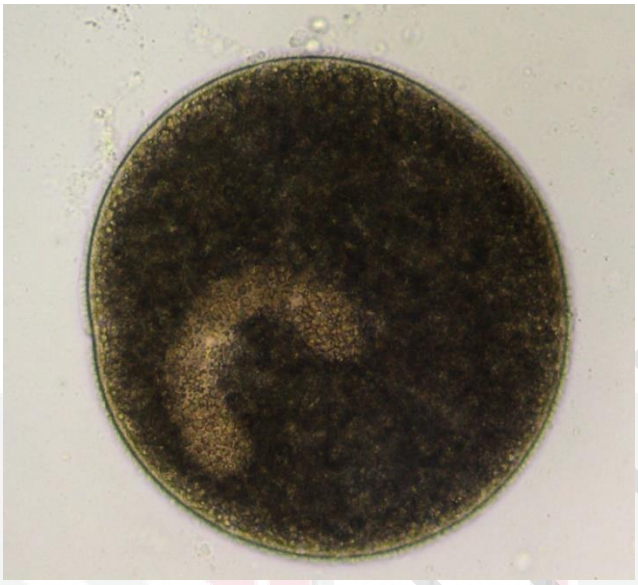

Figure 4:Ichthyophthirius multifilis (Dickerson, 2012).

2.5 Mycobacteriosis

Mycobacteriosis, a preeminent and prevalent bacterial disease among fishes, is orchestrated by *Mycobacterium* species a group of gram-positive, nonmotile, pleomorphic bacilli with pronounced acid-fast staining properties. As highlighted by Novotny et al. (2004), *Mycobacterium marinum*, *M. fortuitum*, and *M. chelonae* emerge as prominent culprits behind fish mycobacteriosis, a disease particularly notorious for its impact on tropical pet fish, yielding elevated morbidity and mortality rates (Chang and Whipps, 2015).

Emphasizing susceptibility, Astrofsky et al. (2000) assert that freshwater fishes from the families Anabantidae, Characidae, and Cyprinidae are particularly prone to *Mycobacterium* infection.

Clinical manifestations in infected fish span a spectrum, ranging from abdominal swelling to emaciation and uncoordinated swimming. Gross lesions manifest in diverse forms, encompassing skin ulceration to tumour-like nodules afflicting visceral organs, with the spleen, kidney, and liver being most commonly affected (Hashish et al., 2018).

Transmission dynamics underscore the ease with which mycobacteriosis proliferates, disseminating through exposure or direct inoculation via wounds or any available opening. The incubation period, spanning weeks to months, heralds a gradual manifestation of symptoms. This latent phase precipitates a decline in fish health, culminating in emaciation and impaired swimming abilities (Pate et al., 2005). A diminished growth rate or heightened mortality becomes apparent during this period, with certain individuals surviving for years despite chronic mycobacteriosis.

Postmortem examinations reveal classic granulomatous lesions in visceral organs, as eloquently documented by Spickler Anna Rovid and Dvorak Glenda (2020). The intricate interplay between Mycobacterium species and susceptible fish hosts warrants further exploration to unveil potential avenues for disease management and prevention.

2.5.1 Zoonotic potential

Mycobacterium sp. has garnered attention for its potential to cause zoonotic infections in humans. Mycobacteriosis, often referred to as "the fish handler's disease," is notably prevalent among individuals involved in the seafood industry

and those maintaining fish as a hobby. Human infections primarily affect the skin, particularly in areas that have been in contact with contaminated water, such as the knee, elbow, and foot. Skin lesions associated with mycobacteriosis in humans typically manifest as localized papulopustular lesions that may form clusters (Spickler Anna Rovid & Dvorak Glenda, 2020).

In recent years, there has been a decline in reported cases of this zoonotic disease. This decrease may be attributed to the growing awareness and education among individuals regarding the disease. Notably, in Malaysia, despite mycobacteriosis being highlighted as a common bacterial disease in shrimp farms (Hashish et al., 2018), there have been no reported outbreaks in humans. This underscores the importance of continued education and vigilance in preventing and managing potential zoonotic infections associated with *Mycobacterium* sp.

2.6 Epizootic ulcerative syndrome

Epizootic ulcerative syndrome (EUS) stands as a seasonally recurrent and perilous disease afflicting freshwater fishes, garnering recognition on the OIE list. Since its initial outbreak in 1972, EUS has emerged as a pervasive global concern, exerting a profound impact on both wild and farmed fish populations and significantly affecting the livelihoods of fishermen. The causative agent, *Aphanomyces invadans*, an aquatic fungus belonging to the family Saprolegniaecea, exhibits remarkable invasiveness and proliferative tendencies. Even a minimal infection of fewer than 10 zoospores can trigger extensive damage across multiple organs, ultimately leading to fish mortality.

Distinctive manifestations of EUS lesions encompass severe dermal and muscle ulceration. Interestingly, early lesions in EUS are characterized by erythematous dermatitis with no apparent oomycete involvement. The OIE has not identified *B. imbellis* as susceptible to EUS. However, due to being an anabantid, *B. imbellis* might be susceptible to EUS.

Since the inaugural outbreak in Japan in 1971, EUS has traversed borders, affecting countries such as Australia, Singapore (Roberts et al., 1989), Bangladesh, Malaysia (Lilley et al., 1998), and others. Notably, confirmed outbreaks in Botswana, Namibia, and Zambia were documented in 2007 (FAO, 2009). To date, EUS outbreaks have been reported in over 20 countries across North America, Southern Africa, Asia, and Australia (see Figure 5). While Malaysia has not witnessed EUS cases since the 1980 outbreak in Bekok River, Johor, recent reports hint at the emergence of new cases in the northern region of Peninsular Malaysia.

This exploration sheds light on the multifaceted challenges posed by EUS, emphasizing the need for global collaboration in understanding, preventing, and managing this impactful disease.

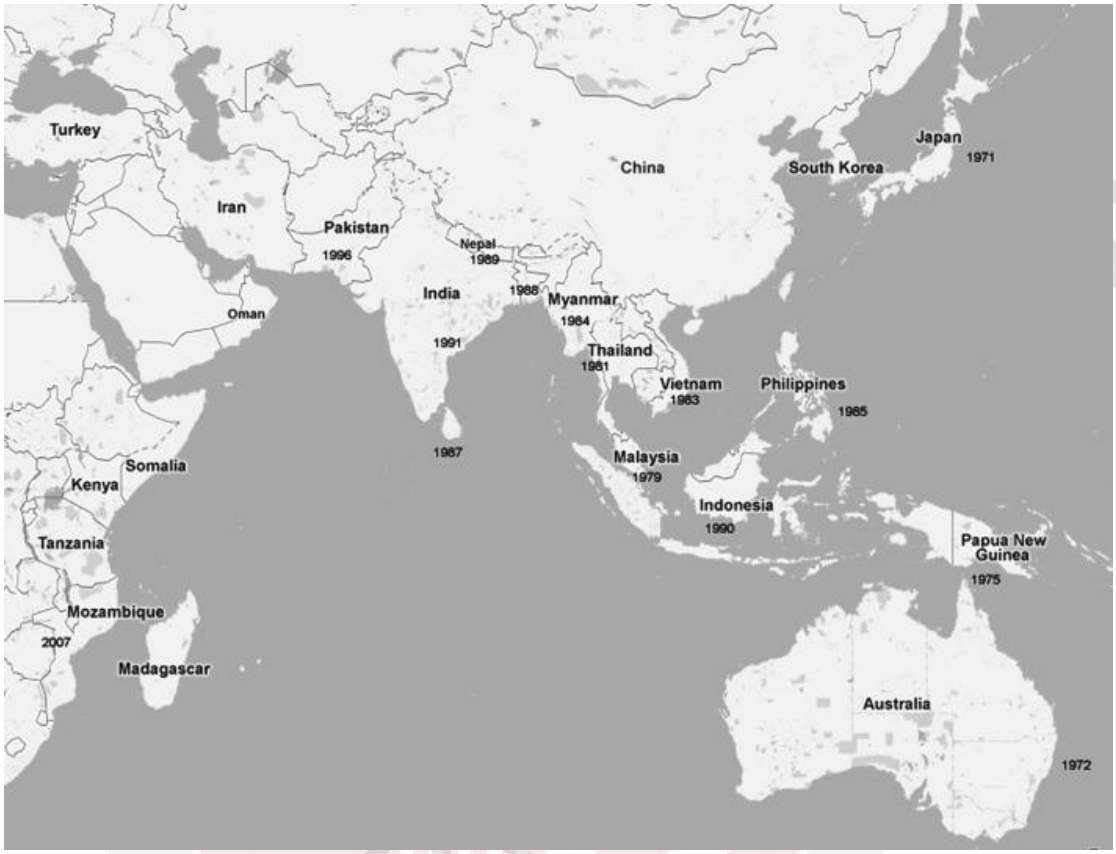

Figure 5:Map showing the geographical spread of EUS in the last three decades (John & George, 2012).

3.0 METHODOLOGY

3.1 IACUC Approval

This study was conducted upon approval of the IACUC reference code UPM/IACUC/AUP-U005/2023.

3.2 Sample collection

Twenty adult *Betta imbellis* were gently captured using scoop nets from the marshes in Kapar, a town nestled in Klang, Selangor. The collection involved two distinct groups of ten adults each, procured from two separate sites; Location 1 (coordinates 3°12'29.847"N 101°24'1.817"E) and Location 2 (coordinates 3°12'15.614"N 101°24'44.938"E). The captured fish were then carefully transported to the Aquatic Animal Health Unit (AAHU) at Universiti Putra Malaysia for subsequent processing.

Ensuring ethical treatment, all fish underwent humane euthanasia using the cervical transection technique. Subsequently, a thorough examination was conducted to identify any potential clinical manifestations. This included the observation and recording of indicators such as abdominal distension, exophthalmia, emaciation, visible skin lesions, or any other abnormalities that may be present.

3.3 *In-situ* water quality

The water quality of the swamp area was assessed using a YSI probe meter, with key parameters such as temperature, pH, and dissolved oxygen measured and recorded.

3.4 Ectoparasitic examination (Piscinoodiniasis and ichthyophthiriasis)

The fish underwent a thorough examination to detect gross lesions characterized by white spots or a velvety gold-dusted appearance. Wet mount sampling from various areas including the body surface, fins, and excised gills was conducted. Scrapings from the skin were obtained using the blunt side of a scalpel blade #20, and the mucus was then spread onto a glass slide for microscopic analysis under a light microscope. Subsequently, the gills were scrutinized for any potential lesions. Those displaying white spot lesions were carefully collected for further microscopic examination, following established protocols (Frimeth, 1994; Noor-Shahirah et al., 2018). The distinctive round body with a large crescent-shaped nucleus characteristic of the agent *Ichthyophthirius multifilis* and the round body and starch granules of *Piscinoodinium* sp. was identified and recorded.

3.5 Bacterial examination (Mycobacteriosis)

All specimens underwent dissection for the exposure of abdominal organs. Internal organs, including the liver, spleen, and kidney, were meticulously examined for the presence of granulomatous lesions characterized by small, creamy-white miliary nodules. Organs exhibiting these distinctive lesions were identified for further

examination. Smears originating directly from the affected organs underwent Ziehl-Neelsen acid-fast staining, facilitating microscopic examination. Concurrently, fish lacking typical lesions were selected for histopathological sampling to elucidate the potential presence of microgranulomas.

3.6 Fungal examination (Epizootic ulcerative syndrome)

The fish underwent examination to identify any visible abnormalities. Individuals displaying ulcerative lesions or red spots on their body surface were chosen for skin scraping, and the obtained samples were stained using Grocott's stain. In contrast, fish without apparent lesions were identified for histopathological sampling. All prepared slides underwent staining with haematoxylin and eosin (H&E). The presence of the agent, distinguished by basophilic hyphae surrounded by granulomas, was carefully documented. Additionally, any histological alterations in the muscle tissue were noted.

3.7 Data analysis

3.7.1 Prevalence of disease

Disease prevalence was calculated using the following formula (Noor-Shahirah et al.,2018):

$$\left(\frac{No.of\ diseased\ fish}{No.of\ sample\ population}\right) x\ 100$$

3.7.2 Statistical analysis

Statistical analyses were carried out to determine the significance of the prevalence association between two populations of wild *B. imbellis*. Fisher's exact test was used with a significant value ($p<0.05$) and a confidence interval of 95%.

4.0 RESULT

4.1 Gross lesions

In this study, a total of 20 fish were examined for the presence of typical lesions on the body surface and visceral organs. All 20 fish showed no gross lesions on the body surface, 20 fish showed no significant lesions on the skin or fins (Figure 6) and no lesions on the visceral organs (Figure 7).

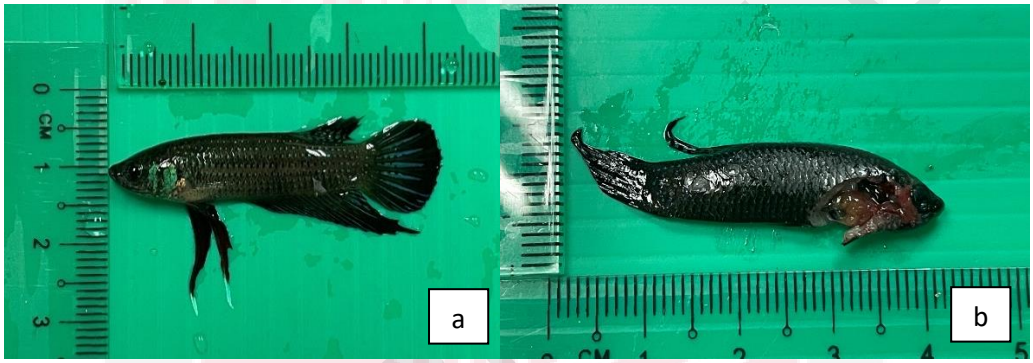

Figure 6: B. imbellis with no external (a) or internal (b) lesions

Figure 7: B. imbellis visceral organs showing no gross lesions.

4.2 Wet mount findings

Skin scrapings and gill wet mount from 20 fish were collected for analysis to determine the presence of *Ichthyophthirius multifiliis* and *Piscinoodinium* spp. All smears revealed no presence of agents (Figure 8).

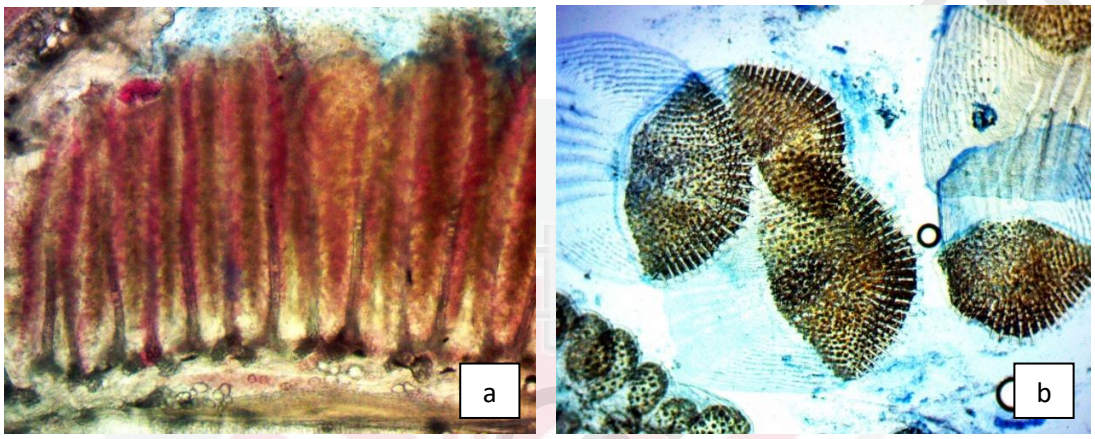

Figure 8: Gill wet mount (a) and skin scrapings (b) showing no presence of Ichthyophthirius multifiliis or Piscinoodinium spp.

4.3 Histopathological findings

Histological evaluation (Figure 9) of gills (a) show no presence of *Ichthyophthirius multifiliis* and *Piscinoodinium* spp. as well as no hyperplasia of the primary and secondary lamellae. Muscle sections (b) show no presence of necrosis or fungal hyphae indicating complete absence *Aphanomyces invadans*. The histological evaluation of the liver (c) and kidney (d) show no presence of granulomas or micro-granulomas indicating the absence of mycobacteriosis.

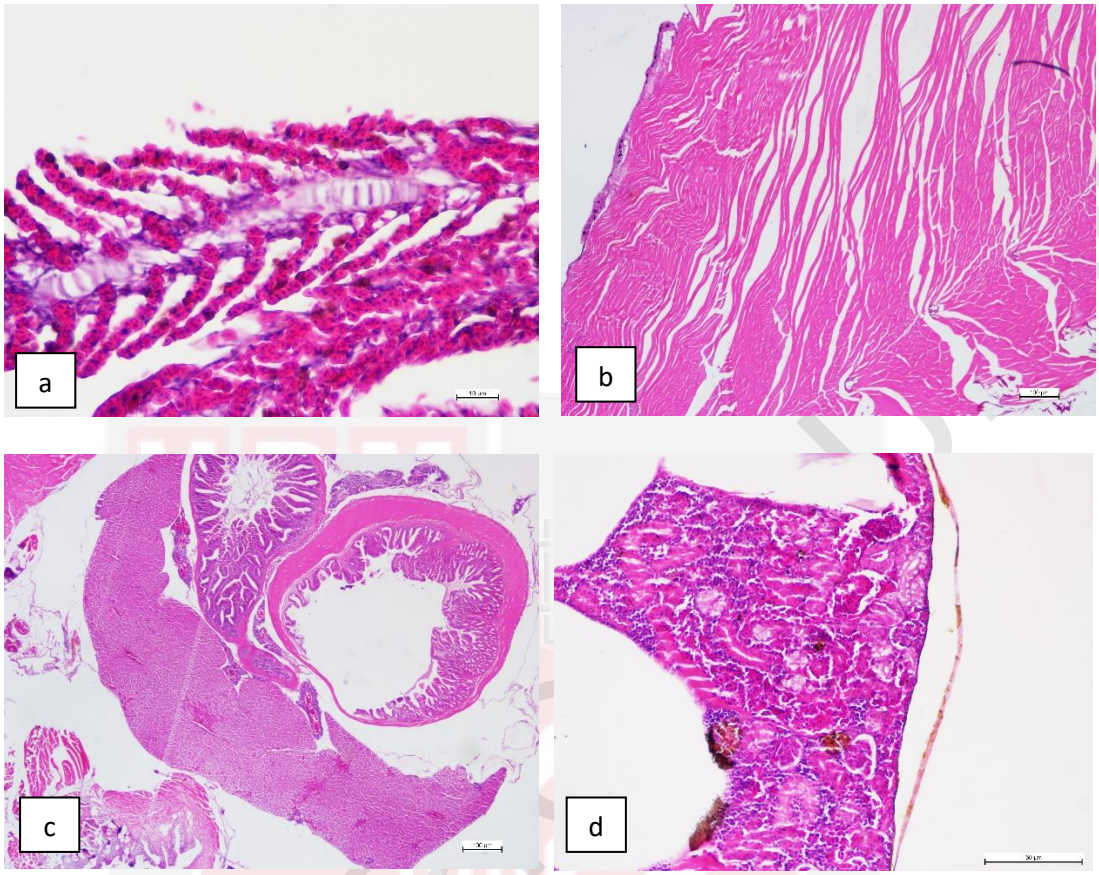

Figure 9: Histological slides of gills (a), skeletal muscle (b), liver (c), and kidney (d)

4.4 Prevalence of disease

The prevalence of piscinoodiniasis, ichthyophthiriasis, mycobacteriosis, and epizootic ulcerative syndrome is presented in Table 1. No specimens examined tested positive for any of the diseases. This makes the prevalence of these four diseases zero.

Location	**1**	**2**
Piscinoodiniasis	0	0
Ichthyophthiriasis	0	0
Mycobacteriosis	0	0
Epizootic ulcerative syndrome	0	0

Table 1: Prevalence of piscinoodiniasis, ichthyophthiriasis, mycobacteriosis and epizootic ulcerative syndrome.

5.0 DISCUSSION

The investigation into disease prevalence and the correlation between two populations of wild *B. imbellis* in Klang yielded promising results, revealing no significant variations between them. This suggests a reassuring scenario where the wild *B. imbellis* population in Klang appears to be resilient against the four prevalent diseases: piscinoodiniasis, ichthyophthiriasis, mycobacteriosis, and epizootic ulcerative syndrome. However, it's crucial to acknowledge that environmental conditions play a pivotal role in shaping these findings.

The water samples collected during the sampling process exhibited a moderate level of acidity, with readings of 4.23 at Location 1 and 4.3 at Location 2. The lower pH in the soil could stem from natural acidity or disturbances caused by agricultural or residential development, both factors contributing to a reduction in soil and water pH. This acidity, in turn, affects the survival of pathogens. The pH values at the sampling site were outside the optimal range for pathogens such as *Ichthyophthirius multifiliis* and *Mycobacterium* species, suggesting their potential absence in the water samples.

Another noteworthy factor is the seasonal pattern, which encompasses wet and dry seasons with varying precipitation frequencies. The dry season, occurring from May to September in Malaysia, aligns with the sampling period in August. *Aphanomyces invadans*, known to sporulate favourably in response to heavy rainfall, might not have been as prevalent during the dry season.

Additionally, the absence of disease prevalence could be linked to the possibility that infected fish succumbed to mortality or became prey for other predators. Abnormal behaviors exhibited by infected fish, such as uncoordinated swimming patterns, render them more susceptible to predation. Natural predators in the ecosystem play a vital role in controlling the population of diseased fish, consuming infected prey and reducing the likelihood of disease spread.

Histopathological examination of tissue samples uncovered the presence of melanomacrophages. The study site, Klang, known for its acidic sulfate soils along the coastal region, potentially explains the heightened iron uptake observed in the soft tissue of the samples. This aspect adds a layer to the understanding of the ecological dynamics influencing the health of the *B. imbellis* population in the region. In conclusion, this multifaceted study provides valuable insights into the complex interplay of environmental factors, seasonal patterns, and predator-prey dynamics that contribute to the health status of wild *B. imbellis* in the Klang region.

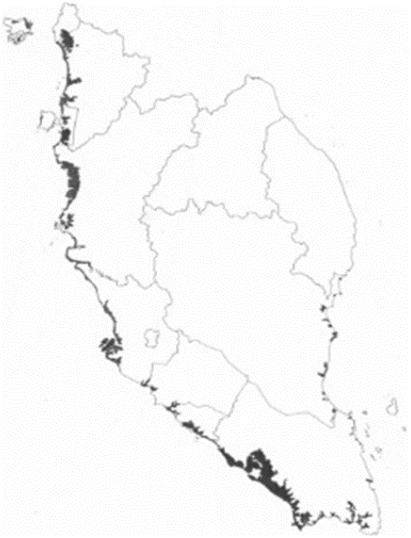

Figure 10: A map of Peninsular Malaysia showing the distribution of acid sulphate soils (shaded area)

6.0 CONCLUSION

In conclusion, there was no evidence of mycobacteriosis, ichthyophthiriasis, piscinoodiniasis, or epizootic ulcerative syndrome in 20 wild *B. imbellis* from Klang, Selangor. Despite the fish's lack of clinical symptoms, the histopathological results indicated the presence of melanomacrophages rather than the presence of agents. Although numerous other potential factors should be investigated, environmental factors may have played a major role in this finding.

7.0 RECOMMENDATION

By conducting studies with a larger sample size and a longer duration, it would be beneficial to expand the breadth and depth of our understanding for future research. With a more thorough understanding of the prevalence of diseases across diverse populations of wild B, this method would help to increase the body of epidemiological data. imbellis. Molecular studies could also be incorporated into the research framework, which could be advantageous. With the molecular perspective these diseases provide, rapid and precise diagnosis can be achieved through the use of techniques like polymerase chain reaction (PCR). In addition, conducting a prospective study would provide us a forward-looking viewpoint and enable us to investigate the evolution of diseases in the wild population of B. imbellis. We may be able to better understand the health dynamics of this fascinating species by using this approach, which may reveal dynamic patterns and illuminate the factors influencing the evolution of disease over time.

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