

## ENDOPARASITES INFECTION IN BLOOD COCKLE (*Anadara granosa*) IN ACEH BESAR WATERS, INDONESIA

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### ABSTRACT

This study aims to investigate the type, prevalence, and intensity of endoparasites infecting the organs of blood cockles (*Anadara granosa*) in the waters of Aceh Besar, Indonesia. A purposive sampling method was conducted from March to August 2020 consisting of two sampling sites, namely the waters of Ujung Pancu and Krueng Raya. Endoparasite identification and analysis was carried out at the Parasite Laboratory of the Fish Quarantine Center, Quality Control and Safety of Fishery Products in Blang Bintang, Aceh Besar. The results showed that there were two types of endoparasites living in the blood cockles: namely, *Perkinsus* sp., which belong to the group Protozoa, and *Spiroxys* sp. of the Nematode class. The prevalence levels of *Perkinsus* sp. was 73.33% and were found in the waters of Ujung Pancu; while the lowest prevalence level was 20% in blood cockles that were infected with *Spiroxys* sp., which were found both in Ujung Pancu and Krueng Raya. The highest intensity level was infections of *Perkinsus* sp. with a value of 89 ind/species in the Krueng Raya site. The lowest intensity value was infections of *Spiroxys* with a value of 2 ind/species in Krueng Raya and 2.3 ind/species in Ujung Pancu

Key words: disease intensity, parasites, prevalence

### ABSTRAK

Penelitian ini bertujuan mengetahui jenis, prevalensi, dan intensitas endoparasit yang menginfeksi organ tubuh kerang darah di perairan Aceh Besar, Indonesia. Penelitian ini dilakukan pada bulan Maret hingga Agustus 2020 dengan metode purposive sampling yang terdiri atas dua lokasi yaitu di perairan Ujung Pancu dan perairan Krueng Raya. Identifikasi dan analisis endoparasit dilakukan di Laboratorium Parasit Balai Karantina Ikan, Pengendalian Mutu dan Keamanan Hasil Perikanan di Blang Bintang, Aceh Besar. Hasil penelitian menunjukkan terdapat dua jenis endoparasit yang menginfeksi organ dalam kerang darah yaitu *Perkinsus* sp. yang termasuk kelompok Protozoa dan *Spiroxys* sp. dari kelas Nematoda. Tingkat prevalensi tertinggi adalah *Perkinsus* sp. sebesar 73.33% yang terdapat di perairan Ujung Pancu sedangkan tingkat prevalensi terendah adalah 20% pada kerang darah yang terinfeksi *Spiroxys* sp baik di Ujung Pancu maupun Krueng Raya. Tingkat intensitas tertinggi adalah dari jenis *Perkinsus* sp. dengan nilai 89 ind/spesies di lokasi Krueng Raya, sedangkan nilai intensitas terendah ditemukan pada *Spiroxys* dengan nilai 2 ind/spesies di perairan Krueng Raya dan 2,3 ind/spesies di perairan Ujung Pancu.

Kata kunci: intensitas penyakit, parasit, prevalensi

### INTRODUCTION

Indonesia is one of the largest marine areas in the world which is famous for its abundant marine resources, in term of both fisheries and crustaceans (Muchlisin *et al.*, 2017; Rizwan *et al.*, 2017; Putra *et al.*, 2018; Muhammadar *et al.*, 2019; Safriani *et al.*, 2019). The blood cockle (*Anadara granosa*) is a type of shellfish that has a high economic value and is cultivated as a source of protein and minerals (Yulinda *et al.*, 2020). There are various types of shellfish that have been recognized by the community and are also used as food ingredients. In addition, blood cockles can also be used as decoration, fertilizer, and for absorption of heavy metals (Solang, 2019). Blood cockles are a type of cockle belonging to the Arcidae family that live in intertidal zones with sandy substrates and originate from mud flats in Southeast Asia, particularly Indonesia, Malaysia, and Thailand (Khalil *et al.*, 2017). Substrates, season, temperature, feed and other chemical factors which vary in each region are very important parameters to support oyster growth (Lindawaty *et al.*, 2016). Thus, blood cockles can also be used as a bio-indicator of water pollution, because the habitat of these animals is at the bottom of the waters and as stated before they accumulate heavy metals (Supriatno and Lelifajri, 2009; Yona *et al.*, 2020). If a high cadmium concentration is found in the

cockles that exceeds the normal limit of this organism, then this can be used as an indicator of pollution in the environment (Hossen *et al.*, 2014).

Several recent studies that have been carried out on multiple blood cockle species. Among them was a study with collected data on blood cockles found in coastal waters and aquaculture ponds in the Gulf of Thailand (Theerachat and Glinwong, 2020). Other studies included descriptions and challenges of blood cockle cultivation in Malaysia (Saffian *et al.*, 2020), microplastic concentrations in blood cockles found in Pandeglang Banten Indonesia (Ukhrowi *et al.*, 2021), the concentration of heavy metals in blood cockles found on the coast of East Java and potential risks to human health (Soegianto *et al.*, 2020), the health status of blood cockles in cultivation ponds in Malacca Strait (Mohamat-Yusuff *et al.*, 2020), the economic potential of blood cockles in Riau province (Yulinda *et al.*, 2020), and lastly studies on the bio-ecological aspects and reproductive biology of blood cockles (Khalil *et al.*, 2017; Sulistyaningsih and Arbi, 2020). Parasites are a type of infectious disease that attack many fishery commodities both in nature and in cultivated environments. In its application, parasites attack aquatic organisms with the principle of utilizing their host (Abowei *et al.*, 2011). Parasites that are found outside the fish body are called ectoparasites and parasites inside the fish's body are called endoparasites

(Sarjito *et al.*, 2013). Apart from infecting teleost fish, parasites also infect various aquatic organisms such as bivalves, crustaceans, and mollusks (Wood and Lafferty, 2015).

Currently, research on parasites in blood cockles is still very limited. However, parasitic infections in blood cockles in Central Java and the Malacca Strait have been reported (Uddin *et al.*, 2011; Karnisa *et al.*, 2019). Parasitic infection studies in other mollusks have also been reported such as the green-lipped mussel (Trottier *et al.*, 2012; Trottier and Jeffs, 2015), and oysters (Moss *et al.*, 2008; Arzul *et al.*, 2011; Ben-Horin *et al.*, 2018). At present, research on endoparasite infections in blood cockles in the Aceh Besar District has not yet been carried out. Research on endoparasites in blood cockles is very important as it helps to provide a more comprehensive literature on endoparasite infections. Therefore, this study aims to determine the type, prevalence, and intensity of endoparasite infections in blood cockles in Aceh Besar.

## MATERIALS AND METHODS

This research was conducted for 2 months from March to August 2020. Blood cockles (*Anadara granosa*) were obtained from field collection in waters surrounding the Aceh Besar Regency. Sampling was carried out in two locations: namely, Ujung Pancu and Krueg Raya. The identification and observation of blood cockle (*Anadara granosa*) endoparasites was carried out at the Parasite Laboratory of the Fish Quarantine Center, Quality Control and Safety of Fishery Products in Blang Bintang, Aceh Besar.

### Sampling Method

The research method used was a survey method by looking directly at the conditions of the waters at the research location and sampling using a purposive sampling method. Sampling took place in Aceh Besar District, Aceh, Indonesia at two locations. The number of samples taken per location was 15 individuals (Mardiana, 2014). The samples that had been taken were then collected in a bucket for further parasite examination in the laboratory.

Sampling was carried out three times a week for four weeks. The sample was put into a bucket then transported to the parasite laboratory of the Fish Quarantine Center, Quality Control and Safety of Fishery Products in Blang Bintang, Aceh Besar District for identification and analysis. The water quality was first measured before sampling, which includes taking the temperature using a thermometer; the degree of acidity was measured using a pH meter, dissolved oxygen using a DO meter, and salinity using a refractometer.

The blood cockles observed were transported from the container using a gutter and placed in a tray. Then the shells were opened and the targeted organs were cut off to be observed on a microscope. Observation of endoparasites were made on the blood cockles' gills, mantles, and gonads. The organs would first be

dissected and then placed on a glass object that had been lubricated with distilled water as much as 1-2 drops and then observed under a microscope. Parasites were recorded and identified using the parasite manual book (Kabata, 1985).

### Data Analysis

#### Prevalence

Measurement of attack level (prevalence), according to (Fernando, 1972) as follows:

$$\text{Prevalence (\%)} = \frac{N}{n} \times 100$$

Prevalence = Percentage of shellfish attacked by disease (%)

N = Number of shellfish samples attacked by parasites (ind)

n = The number of samples observed

#### Intensity

Measuring the intensity of parasite attacks according to (Fernando, 1972) as follows :

$$\text{Int} \left( \frac{\text{Individual}}{\text{Species}} \right) = \frac{\sum P}{n}$$

Intensity = Intensity of disease attack (individual/species)

$\sum P$  = Total number of parasites

n = The number of samples observed

## RESULTS AND DISCUSSION

### Identification and Clinical Symptoms Of Endoparasites in Blood Cockles

Two endoparasites that were identified in the blood cockles (*Anadara granosa*) from two sampling locations in Aceh Besar were *Perkinsus* sp., and *Spiroxys* sp. Some endoparasites like *Perkinsus* sp., that were found still in the cyst stage which is commonly called a *Perkinsus* sp. cyst. The various endoparasites found in blood cockles within the sampling area is presented in Figure 1.

Clinical symptoms found in blood cockles infected with common parasites showed signs such as gills that were drizzled and the color of the gills looked pale while those in uninfected blood cockles had bright gill characteristics (Nainggolan, 2016). According to (Karnisa *et al.*, 2019) the clinical symptoms of *Perkinsus* sp. infection in blood cockles are an imperfectly shaped shell and the presence of excessive mucus. Other symptoms as reported by (Baker *et al.*, 2007) are blisters on the inner surface of the skin, sores on the mantle, pale flesh color, and excessive mucus. However, infection in blood cockles does not always cause these clinical symptoms. Interestingly, in this study, it found that blood cockles that were infected with endoparasites did not show any clinical symptoms. Moreover, this is in accordance with (Sarjito *et al.*, 2013) who expressed that an infection of parasitic

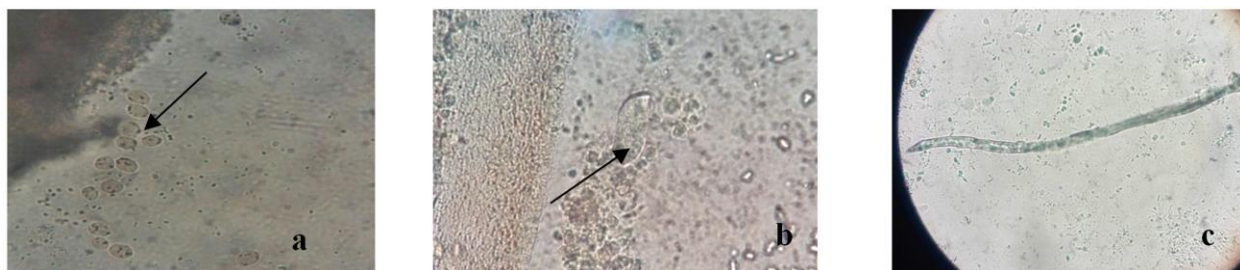
worms does not show clear external clinical symptoms so that it cannot be detected quickly.

**Infected Organ**

In this study, we have examined endoparasite infections on organs in the blood cockle such as the gills, mantle, and gonads as presented in table 1. The most common sites for endoparasite infection are the gills, followed by the mantle and gonads. This finding was in accordance with a study by Elston et al. (2003) who reported a finding of parasite infection in Manila cockles (*Venerupis philippinarum*). They also found that *Perkinsus* sp. is usually found on the gills, mantle, and palp. A similar finding was observed in waters surrounding Jakarta and Central Java that also reported that the dominant parasitic infection in blood cockles there was *Perkinsus* sp. (Choi and Park, 2010; Karnisa et al., 2019). However different results were found in blood cockles infected with endoparasites in the

Malacca Strait. They reported the organs that were frequently affected by endoparasites were the gonads, followed by the stomach epithelium, mantle, digestive glands, and foot muscle (Uddin et al., 2011).

Apart from *Perkinsus*, the endoparasite that was also found in this study was *Spiroxyx* sp. *Spiroxyx* sp. is in the nematode class of endoparasite that infects almost all species of aquatic biota. In several reports, *Spiroxyx* sp. has also been found in several freshwater fish (Santos et al., 2009; Galaviz-Silva et al., 2013; Vieira-Menezes et al., 2017; Mhaisen et al., 2018; Šimková et al., 2019), farmed fish (Soler-Jiménez et al., 2017), turtles (Rakhshandehroo et al., 2020) and some marine fishes (Öktener, 2016). However, in this study, *Spiroxyx* sp was also found in the blood cockles' gonads. The characteristics of *Spiroxyx* sp., found in the blood cockles samples had a non-segmented body shape and had a pointed tip. *Spiroxyx* sp. larvae are motile as they are capable of active movement. once



**Figure 1.** Endoparasites infected blood cockles (*Anadara granosa*). a= *Perkinsus* sp., b= *Perkinsus* sp., cyst, c= *Spiroxyx* sp.

**Table 1.** Endoparasites in blood cockles (*Anadara granosa*) based on sampling site

Site	Parasite name	The number of parasites on the target organ		
		Gill	Mantle	Gonad
Ujung Pancu	<i>Perkinsus</i> sp.	624	317	-
	<i>Perkinsus</i> sp. cyst	28	23	-
	<i>Spiroxyx</i> sp.	-	-	7
Krueng Raya	<i>Perkinsus</i> sp.	628	262	-
	<i>Perkinsus</i> sp. Cyst	59	55	-
	<i>Spiroxyx</i> sp.	-	-	6

**Table 2.** Prevalence and Intensity in blood cockle (*Anadara granosa*).

Site	Parasite	∑ Parasite	∑ Parasite samples	∑ Samples	I	P (%)
Ujung Pancu	<i>Perkinsus</i> sp.	941	11	15	85.54	73.3
	<i>Perkinsus</i> sp. cyst	51	10	15	5.1	66.7
	<i>Spiroxyx</i> sp.	7	3	15	2.3	20
Krueng Raya	<i>Perkinsus</i> sp.	890	10	15	89	66.7
	<i>Perkinsus</i> sp cyst	114	9	15	12.6	60
	<i>Spiroxyx</i> sp.	6	3	15	2	20

I= Intensity, P= Prevalence

**Table 3.** Water quality parameters in sampling sites

Sites	Parameter	Unit	Measurement results	Range	Reference
Ujung Pancu	pH	-	7.5-7.8	6.5-8.5	(Mayunar and Purwanto, 1995)
	Salinity	ppt	30-35	> 25	(Broom, 1985)
	Temperature	°C	26-29	25-30	(Broom, 1985)
	DO	ppm	3.9-4	4.9-6.4	(Atmaja et al., 2014)
Krueng Raya	pH	-	7.3-7.5	6.5-8.5	(Mayunar and Purwanto, 1995)
	Salinity	ppt	28-30	> 25	(Broom, 1985)
	Temperature	°C	26-29	25-30	(Broom, 1985)
	DO	ppm	3-4	4.9-6.4	(Atmaja et al., 2014)

these larvae reach the adult phase, they can be commonly found in marine mammals, such as dolphins and whales, and live within their muscle tissue (Tamba *et al.*, 2012). The identification of this parasite is in accordance with the research of (Uddin *et al.*, 2011) who also found parasites in blood cockles. However, the parasites detected in the gonadaltissue of these blood cockles were Turbellarian (Bower *et al.*, 1994) stated that parasites that reside in the gonads can cause interference with gametogenesis and result in failure to reproduce.

Besides infecting gonads, some endoparasites also attack the gill and mantle area. Gills and mantles are the main target organs of *Perkinsus* sp. infection (Choi and Park, 2010). The gills on shellfish have a way of feeding by filtering (filter feeder) organic matter suspended in the waters using siphons. Another function of the gills of a shellfish can be as an organ for gas exchange or as a means of breathing. Shellfish can select food particles to be filtered and consumed, this is because each shellfish siphon has different sensors and behaviors towards food (Bachok *et al.*, 2006). This was confirmed by (Allam *et al.*, 2013) which stated that *Perkinsus* sp. cells live and swim freely in the waters, and are pumped into the pallial cavity and then captured within the gills. *Perkinsus* sp. develop and undergo incubation for three days inside the gills, then move on to infect other tissues. Parasitic cells enter the mantle cavity which will then develop and the remaining parasites that are not swallowed will be discharged as feces. The rest of these feces will be contagious towards healthy shellfish.

### Intensity and Prevalence

The intensity and prevalence of blood cockle endoparasite in Aceh Besar have been presented in table 2. In general, the level of prevalence and intensity of each sampling site in Aceh Besar was different; the prevalence value of blood cockles in Ujung Pancu was higher than in Krueung Raya. This is assumed to be due to the existence of residential areas, stalls, and also fishing industry activities such as salted fish and octopuses that are very close to these waters. Therefore as a result of water pollution, this may stimulate parasite infection. However, differences in the prevalence and intensity levels of parasites from one area to another are often related to many different factors, including: spatial variations, population densities of the animals and the presence of alternative hosts (Uddin *et al.*, 2011). Meanwhile, according to (Nainggolan, 2016), the difference in prevalence at each location is due to the factors that influence it: for example, the differences in water quality conditions at each shellfish fishing location, the distribution pattern of endoparasites, and also blood cockle immunity. The incubation period of *Perkinsus* sp. for blood cockle infections at each location has a different period as well.

Based on Table 2, the highest prevalence value of *Perkinsus* sp. found in Ujung Pancu was 73.33%, which is categorized as the usual amount which means

that the level of infection is common (Nandlal and Pickering, 2004), moreover, the prevalence value of *Perkinsus* sp. in Krueng Raya was 66.7% which belongs to a very frequent category where the prevalence level can be monitored. However, the intensity value in Ujung Pancu was 85.54 ind/species and in Krueng Raya was 89 ind/species which means that these two values are included in the very heavy category.

The prevalence value of *Spiroxys* in the waters of Ujung Pancu and Krueung Raya showed the same value of (20%) which is included in the frequent category which means that the level of infection often occurs (Nandlal and Pickering, 2004). The intensity value in Ujung Pancu was 2.3 ind/species, which is included in the light category. The intensity value of *Spiroxys* sp attacking blood cockles in Krueung Raya is 2 ind/species which is also included in the light category. A high stocking density can also accelerate the spread of parasites. Apart from the stress factor due to a high density, poor nutrition, and poor water quality, allow for the rapid development of parasites (Anshary, 2010; Anshary *et al.*, 2013).

### CONCLUSION

Two identified endoparasites were found to be infecting blood cockles at Ujung Pancu and Krueng Raya, namely *Spiroxys* sp. and *Perkinsus* sp., (including cysts *Perkinsus* sp.). The highest prevalence value was obtained in *Perkinsus* sp. infections with a value of 73% included in the usual category, while the lowest prevalence rate was *Spiroxys* sp. with a value of 20% included in the frequent category. The highest intensity level was obtained in *Perkinsus* sp with a value of 94.1 ind/species, which was included in the very heavy category, while the lowest intensity level was found in *Spiroxys* sp. with a value of 2 ind/species which belongs in the light category.

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