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Short Communication

First report of *Anisakis sp.* in *Epinephelus sp.* in East Indonesia

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ABSTRACT

Objective: The present research was conducted to identify the prevalence of *Anisakis sp.* as fish-borne zoonoses in *Epinephelus sp.* in territorial waters of East Nusa Tenggara, Indonesia.
Materials and methods: A total of 50 fish (*Epinephelus sp.*) were collected from Kupang Fish Market in East Nusa Tenggara. Identification of *Anisakis sp.* was performed based on morphological observations considering shape of ventriculus, boring tooth, and mucron using binocular microscope.
Results: Prevalence of *Anisakis sp.* in *Epinephelus sp.* was 22% (n=11/50). The parasite was mostly found in abdominal cavity, gonad, intestines, and muscles of fish.
Conclusion: This study suggests that *Anisakis sp.* is present in *Epinephelus sp.*, exerting potential health hazards for human who consume the fish.

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KEYWORDS

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INTRODUCTION

Anisakiasis is fish-borne parasitic zoonoses for human found in most parts in development countries (Audicana and Kennedy, 2008; Ivanovic et al., 2015). Two important parasitic species of Anisakis pathogens are involved in this zoonoses namely *Anisakis simplex* and *A. pegreffii* (Mattiucci et al., 2013; Cipriani et al., 2016; Bao et al., 2017). It is also known that *A. simplex* can cause allergies to human (Ivanovic et al., 2015). Anisakiasis is considered as a foodborne disease; thus, it is important in the view point of trading of sea foods (Šimat et al., 2015). The disease can show early symptoms as sensitization to allergic and chronic conditions in humans (Baird et al., 2014).

The main source of anisakiasis infection in humans is the raw fish containing Anisakis larvae (Acha and Szyfres, 2003). *A. simplex* can cause severe hypersensitivity reactions and an anaphylactic reaction due to exposure to allergens derived from *Anisakis sp.* (Audicana and Kennedy, 2008). Other clinical symptoms caused by *Anisakis sp.* are gastrointestinal disorders and abdominal pain (Vaughan et al., 2015) as well as adhesive intestinal obstruction and asymptomatic colonic disorders (Takamizawa and Kobayashi, 2015; Tamai and Kobayashi, 2015). Several species of fishes have been reported to be infected with *Anisakis sp.* include in *Auxis thazar*, *Rastrelliger kanagurta*, *Katsuwonus pelamis* in East Indonesia (Uga et al., 1996; Anshary et al., 2014; Hibur et al., 2016). *Epinephelus sp.* is considered as one of the important fishes in Indonesia (Jefri et al., 2015). The aim of this study was to find the prevalence of *Anisakis sp.* as fish-borne zoonoses in *Epinephelus sp.* in territorial waters of East Nusa Tenggara, Indonesia.

MATERIALS AND METHODS

Samples (n=50) of *Epinephelus sp.* were collected from Kupang Fish Market, East Nusa Tenggara, Indonesia. The fish samples were labelled and placed in coolbox finally delivered for inspection. The research work was conducted at the Parasitology Laboratory, Veterinary Faculty, Nusa Cendana University, and Fish Quarantine Laboratory, Ministry of Marine and Fisheries, Republic of Indonesia. Anisakis larvae examination was conducted after splitting the fishes using scissors, and all the internal organs were examined under microscope. The laterally discovered Anisakis larvae were cleaned for the identification on the anterior and posterior. The offal or parts of the organs in the examined fish included muscle, liver, spleen, and the intestine. The organ was placed in a petridish and added physiological solution on it, and then examined the presence of *Anisakis sp.*

Identification of *Anisakis sp.* was conducted based on morphological observation of ventriculus, boring tooth, and mucron using binocular microscope. According to Shiraki in 1974, the genus *Anisakis* was classified based on the specific characteristics of Type I and Type II (D'Amelio et al., 2010). The main morphological characteristics in differentiating Anisakis types were excretory pore, mucron, venticulus and boring tooth (Fukuda et al., 1988) and the determination of prevalence was based on morphological identification, as described by Castellanos et al. (2017).

RESULTS AND DISCUSSION

Based on the results of research on *Epinephelus sp.*, Anisakis larvae Type I and Type II were found in this study. Anisakis larvae Type 1 had the characteristics boring tooth, ventriculus, and mucron located at the tail end (**Figure 1**). *Anisakis sp.* Type II was found to have morphological characteristics to the posterior end of the conical and the absence of mucron (**Figure 2**).

The most distinguishing morphological difference was that Type 1 had a mucron while Type 2 did not have any mucron, as supported by Castellanos et al. (2017). The boring tooth function was found on the anterior part to perforate the intestinal wall. Boring tooth served as a larval defense tool for holding onto the mucosa of the small intestine when there was an intestinal contraction in digesting food (Murata et al., 2011). The third stage had a boring tooth, ventriculus, and posteriorly there was a mucron located on the posterior. Excretory pore served as a discharge hole located between the ventrolateral lips, while the esophagus served as a link between the mouth with the ventriculus. Ventriculus was directly related to the intestine, which is considered as an important characteristic of *Anisakis sp.* and other nematodes (Fukuda et al., 1988).

The posterior part of *Anisakis sp.* larvae had anus and mucron located at the posterior end. The results obtained in *Epinephelus sp.* also found the existence of Anisakis larvae Type II with a total of 5 larvae. Mattiucci and Nascetti (2006) confirmed that Anisakis larvae Type II had the same boring tooth and ventriculus, but did not have a mucron in the posterior. This result was supported by the statement of Castellanos et al. (2017) suggesting that larva Anisakis Type I had mucron, which was absent in Anisakis Type II larva.

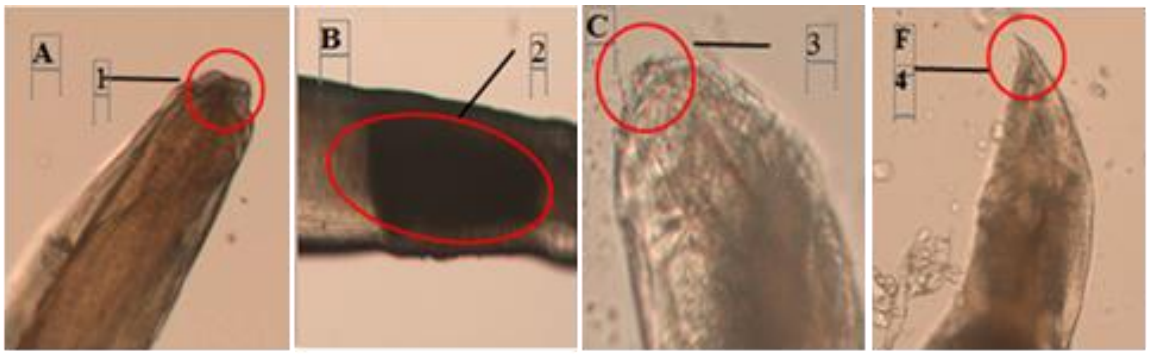

Figure 1. Morphology of anisakis larva type I in *Epinephelus sp*: boring tooth (1); ventriculus (2); mucron (3); tail (4)

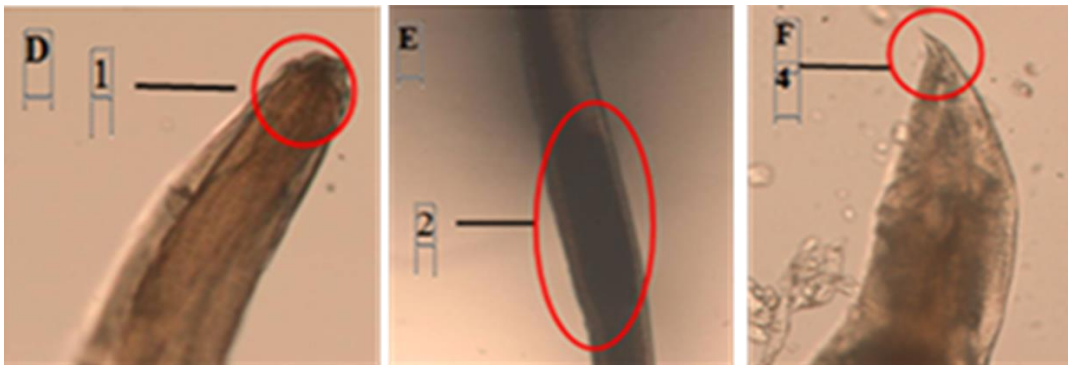

Figure 2. Morphology of anisakis larva Type II in *Epinephelus sp*: boring tooth (1); ventriculus (2); tail (4)

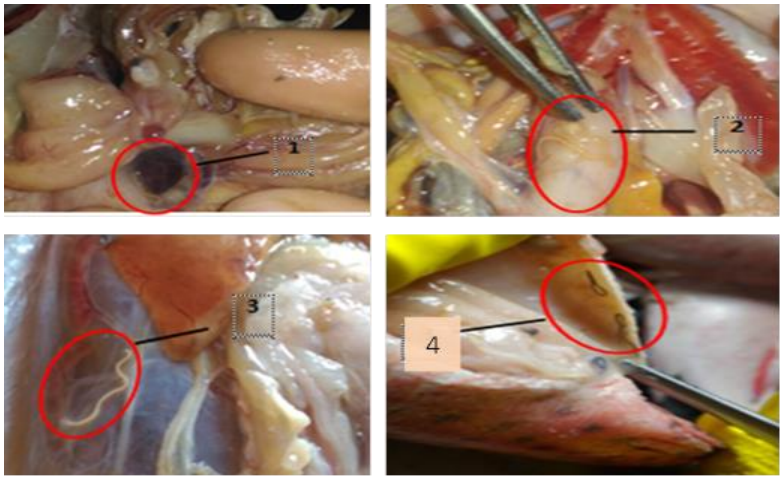

Figure 3. Infected Organs with *Anisakis* larva: abdominal cavity (1); intestines (2), gonad (3), and muscles (4)

The results showed that from 50 samples of *Epinephelus sp*., 11 were identified as Anisakis larvae. This study concluded that the Anisakis prevalence in East Nusa Tenggara waters at *Epinephelus sp*. was 22%. From these 11 positve samples, 22 larvae (L3) were found different parts that included 17 larvae in the abdominal cavity, 1 larva on gonad, 6 larvae on the intestine, and 3 larvae on muscles (**Figure 3**).

Based on the results of this study, several predilection organs Anisakis larvae were established including abdominal, intestinal, gonad and muscle cavities. The predilection organs might be affected by the nutritional and survival factors of *Anisakis sp*. (Mladineo et al., 2016). The risk of zoonoses by parasites from fish usually enters the human body in the form of a third-stage larva (L3) that enters along with the unrefined or uncooked fish eaten, as supported by (Acha and Szyfres, 2003).

An adult nematode of *Anisakis sp.* can be found in the lumen of the marine mammal gut, which subsequently lays eggs that are excreted through feces (Chai et al., 2005). The floating eggs became the larval stages L1 and L2. In particular, larvae (L2) exists in small crustaceans until they reach the infective stage of L3. The L3 infected crustaceans are swallowed by fish, and L3 will predict the muscles and digestive tract of fish. In the final stage, the larvae will reach to L4 if the infected fish is swallowed by the marine mammals, where L4 becomes adult (Castellanos et al., 2017). In the human body, larvae will live and penetrate the intestinal mucosal tissue. Cases of infection are generally menifested by *Anisakis sp.* This study adds data on the incidence of zoonotic diseases in eastern Indonesia for the first time, particularly anisakis prevalence; whereas, some reports on parasite in fish in Indonesia in several fish species (Hadidjaja et al., 1978; Anshary et al., 2014; Detha et al., 2015).

CONCLUSION

Prevalence of *Anisakis sp.* in *Epinephelus sp.* is 22%. The organs that become predilection for *Anisakis* sp are abdominal cavity, gonad, intestines, and muscles.

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CONFLICT OF INTEREST

There is no potential conflict of interest in this study, including in the preparation of the manuscripts and publications of this article.

AUTHORS' CONTRIBUTION

AIRD, DAW, JA, YR, CM perpetrated the prevalence of *Anisakis* sp in *Epinephelus* sp. AIRD also drafted the manuscript. YR and CM were involved in writing up of the manuscript with AIRD, DAW, and JA as well as refined English of the draft. Finally, the manuscript was read and commissioned by all authors.

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