

First Report of Pentastomiasis and the Lung Histopathological Changes in *Ptyas mucosa* in Sidoarjo, East Java, Indonesia

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Abstract. This study aimed to determine the prevalence of pentastomiasis, identify the parasite, and observe the pulmonary histopathology in *Ptyas mucosa* collected from snake slaughter in Sidoarjo (Indonesia). The identification of pentastomids was carried out by Semichen-Acetic Carmine, and histopathological preparations of the lungs were performed to observe the microscopic lesions. The results showed that 65% of the snakes had pentastomiasis, and the parasites were characterized as *Kirichephalus pattoni*. Microscopical examination revealed mild fibrosis in the pulmonary area as a result of pentastomid infestation. This is the first report of some pentastomiasis in *P. mucosa* in Sidoarjo, East Java, Indonesia.

Key words: *Kirichephalus pattoni*, Parasitic Pneumonia, Rat Snake, Zoonosis.

Abbreviations:

Running title: Pentastomiasis in Rat Snake

INTRODUCTION

Exotic animals such as reptiles, especially various types of snakes, are now popular as pets throughout the world (Okulewicz et al., 2014). However, they are also concerned with the increasing animal welfare and health issues through the animal trade in the market (Yudhana et al., 2021). Some species of snake are bred in captivity, while others are caught in the wild, and sold as pets or as bushmeat for consumption (Hardi et al., 2017; Yudhana et al., 2021).

Ptyas mucosa is one of the most common snake species found in Indonesia. This type of snake, known as the Oriental rat snake and Indian rat snake, belongs to the Colubridae family which is widely distributed throughout East, South, and Southeast Asia (Auliya, 2010; Muliya and Bhat, 2016; Yuan et al., 2022). *P. mucosa* is a diurnal and semi-arboreal snake species that lives on forest floors, agricultural fields, wet ground, and in close proximity to human habitats. It feeds on frogs, rats, and other small animals (Chittora et al., 2021).

The meat of *P. mucosa* is traded legally in China. The meat and internal organs are used as fresh or frozen food, the bile and blood are used in traditional medicine, and the skin is exported to several European countries as raw materials for the leather industry. Although it is illegal, most rural communities in Indonesia trade and consume the meat and bile of *P. mucosa* for some health reasons (Auliya, 2010; Pranashinta et al., 2017).

Wildlife meat, however, poses a threat of transmitting infection with novel and exotic pathogens to human hosts (Hardi et al., 2017). Then new zoonotic diseases are likely to emerge consequently. For instance, Bats (Chiroptera) are important reservoirs of several emerging diseases, including Ebola virus disease (EVD), severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS), as well as the current pandemic COVID-19 caused by SARS Coronavirus 2 (Irving et al., 2021). Many types of parasites are also the cause of zoonotic emerging diseases, resulting in a significant public health burden, particularly in tropical countries (Alasil and Abdullah, 2019).

Several parasites found in confined reptiles spend a portion of their life cycle in the respiratory system. The majority of respiratory involvement is asymptomatic and modest, generating mostly localized inflammation, with the exception of instances with excessive parasite loads. However, severe disease can result from high burdens or unusual host-parasite interactions, which is frequently made worse by secondary infections (Comolli, 2021). Reptile-borne pentastomiasis is an unusual tropical disease that is neglected and under-researched in Asia, Africa, and South America (Sulyok et al., 2014).

46 Pentastomiasis is a parasitic zoonosis disease caused by infection with pentastomids, belonging to the subclass
47 Pentastomida, under the phylum Arthropoda (Chittora et al., 2021; Tongtako et al., 2013; WHA, 2019). Pentastomida are
48 also known as tongue worms since the adult phase of the genus *Linguatula* has similarities with the mammalian tongues
49 (Paré, 2008). Adult parasites are usually found in the respiratory tract of reptiles, birds, and mammals, including humans,
50 while their larval forms are in the internal organs of insects, mammals, and amphibians (Paré, 2008; Chen et al., 2010;
51 Kelehear et al., 2014). Some zoonotic pentastomida in snakes are *Armillifer* and *Porocephalus crotali* (Rataj et al., 2011).
52 Humans are infected with pentastomids since they consume raw snake meat or drink water contaminated with parasitic
53 eggs (Ayinmode et al., 2010; Kelehear et al., 2014; Tappe et al., 2016).

54 Pentastomiasis in snakes have been reported in many countries, such as poisonous *Bothrops asper* in Costa Rica
55 (Alvarado et al., 2015), Royal Python snakes in Nigeria (Ayinmode et al., 2010), Rat snakes in Sri Lanka and India
56 (Fernando and Fernando, 2014; Sundar et al., 2015), wild snakes in Australia (Kelehear et al., 2014), various species of
57 snakes in Slovenia and Pakistan (Rataj et al., 2011), *Agkistrodon acutus* and *Python molurus* in China (Chen et al., 2010),
58 and *Crotalus totonacus* in Mexico (Tepos-Ramírez et al., 2022).

59 To the best of our knowledge, there are no reports of pentastomids infections in snakes in Indonesia. Therefore, this
60 study reports the prevalence of pentastomiasis, the identification of pentastomids, and the pulmonary histopathological
61 profiles in Rat snakes (*P. mucosa*) in Sidoarjo Sub-district, East Java, Indonesia.

62 MATERIALS AND METHODS

63 Ethical approval

64 This study was reviewed and approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine,
65 Universitas Airlangga (number 678-KE).

66 Parasites collection

67 The collection was conducted at a snake slaughterhouse in Tulangan District, Sidoarjo Regency, East Java Province,
68 Indonesia. The presence of pentastomid parasites was observed in the respiratory tract of sixty slaughtered *Ptyas mucosa*
69 snakes. Pentastomides were taken and cleaned with distilled water, then stored in sterile plastic pots. The lungs of the *P.*
70 *mucosa* infected with the parasite were also isolated for histopathological preparation.

71 Prevalence calculation

72 The prevalence of pentastomiasis in snake samples was calculated by the following formula:

73
$$\text{Prevalence (\%)} = (\text{The number of snakes infected with pentastomids}) / (\text{Total sample of snakes}) \times 100\%.$$

74 Semichen-acetic carmine staining

75 The collected pentastomids were stained with the Semichen-Acetic Carmine technique (Kuhlmann, 2006). Adult
76 pentastomids were fixed on object glasses and soaked in 5% glycerin alcohol for 24 hours. Then they were soaked in 70%
77 alcohol for five minutes. Subsequently, the object glasses with pentastomids were placed in Carmine alcohol solution (1:2)
78 for eight hours. The pentastomids were soaked in acid alcohol for 10 minutes and moved to base alcohol for 20 minutes.
79 Furthermore, graded dehydration was carried out with 70%, 85% and 95% alcohol for five minutes, respectively. The
80 mounting step was done using Hung's I for 20 minutes. Pentastomids were taken and placed on new clean object glasses.
81 The pentastomids were dripped with Hung's II solution and then covered with a cover glass.

82 Parasite identification

83 Characteristics of the head and body shape and body size of the parasite were identified based on Riley and Self
84 (1980), Almedia et al. (2006), and Christoffersen and De Assis (2013; 2015). Identification of parasite egg size was carried
85 out according to Keegan (1943).

86 The length of the fresh parasites was measured from the anterior edge to the posterior end. The head was observed
87 under a stereo microscope and documented with a Canon Digital Single-Lens Reflex (DSLR) camera. Stained parasites
88 were observed using a light microscope with 40-100× magnification and documented using Miconos Optilab. Some
89 female parasites were incubated in PBS overnight at 37 °C to collect the eggs. The supernatants were centrifuged at 1,500
90 rpm for 10 minutes and the pellet was dripped onto a glass object and observed using a light microscope with 100×
91 magnification. Eggs were measured using Miconos Optilab.

92 Histopathological preparations

93 The lungs of snakes with pentastomid infestation were fixed in 10% formalin. Then graded dehydration was carried out
94 with serial alcohol concentrations (70%, 80%, 90%, 96%, and absolute) and clearing using xylol. Furthermore, paraffin
95 embedding was performed to obtain a solid block of tissue. The tissue blocks were sliced (3 micrometers thick) using a
96 microtome, mounted on an object glass, and stained using Hematoxylin and Eosin. Microscopic examination was
97 performed using Nikon Eclipse Ci.

104 **The prevalence of pentastomiasis in *Ptyas mucosa* snakes**

105 Thirty-nine or 65% of the respiratory tract of 60 were positively infected with adult pentastomids. Totally, 204
 106 pentastomids were collected (Table 1). Pentastomida were found to be strongly attached to the trachea and lungs of snakes
 107 (Figure 1). Some pentastomids even buried their heads in the lungs of snakes. The parasites of all snakes positively
 108 infected with pentastomids were found in the lungs (39 snakes), while only 5 snakes which parasites spread to their
 109 tracheas.

110

111 **Table 1.** Prevalence of pentastomiasis in *Ptyas mucosa* based on site and number of parasites.

Sites	Positive (%)	Number of parasites
Lungs	57% (34 of 60)	2-9 with the average of 5 pentastomida
Trachea	0% (0 of 60)	-
Lungs and Trachea	8% (5 of 60)	7-9 with the average of 8 pentastomida
Total	65% (39 of 60)	204 pentastomida

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113

114 **Figure 1.** Pentastomid infestation in the lungs of *Ptyas mucosa*. The white arrows show areas with raised lesions around the attachment
 115 of pentastomids to the tissue. The black arrows show female pentastomids and the red arrow shows male pentastomids.

116

117 This is the first study to determine the prevalence of pentastomiasis in *Ptyas mucosa* in Sidoarjo, East Java Indonesia.
 118 As shown in our results, pentastomids were present in 39 of 60 (65%) snakes. Based on this finding, the prevalence of
 119 pentastomiasis in *P. mucosa* in this study was quite high. In this case, it was slightly the same as the prevalence of
 120 pentastomiasis in Brazil, 8 of 15 or 53.33% of snakes were positively infected with pentastomid. The high prevalence in
 121 this research was probably caused by the food consumed by the snakes. The type of food is very influential on the
 122 prevalence of infection and the type of pentastomida that infected the snakes (Almeida et al., 2007). *P. mucosa* snakes
 123 usually feed on rats, frogs, toads, lizards, and even smaller non-venomous and smaller snakes (Sidik, 2006). According to
 124 Esslinger (1962), rats, frogs, toads, lizards, and even non-venomous snakes can act as the intermediate hosts of
 125 pentastomida.

126

127 **Identification of pentastomida**

128 The characteristics of pentastomid found in this study can be seen in Table 2 and Figure 2. Pentastomids have a long
 129 cylindrical body with a larger head, a flat shape on the ventral, and a tapered tail or posterior. Their body was soft and
 130 whitish with a colorless cuticle, which tends to be transparent so that the internal organs are visible. There were annuli
 131 throughout their body up to the posterior. There are two pairs of hooks (a total of four hooks) around the head that are used
 132 to attach to the host's lung tissue, and it had an ovoid-shaped mouth between the two pairs of hooks.

133

134 **Table 2.** The characteristics of pentastomida compared with *Kiricephalus pattoni* by Riley and Self (1980) and Keegan
 135 (1943).

Parameters	The current study		British Museum collection (Riley and Self, 1980)		Keegan, 1943
	Females	Males	Females	Males	
Total number of parasites	183	21	17	2	-
Body length	72-125 mm (\bar{X} 94.5 mm)	17-28.5 mm (\bar{X} 23.75 mm)	83-137 mm (\bar{X} 100 mm)	28-29 mm (\bar{X} 28.5 mm)	-
Number of	29-41	28-35	34-38	31-32	-

<i>annuli</i>	(\bar{X} 31.6)	(\bar{X} 31)	(\bar{X} 36.5)	(\bar{X} 31.5)	
Diameter of cephalothorax	4-5.5 mm (\bar{X} 4.75 mm)	2-2.5 mm (\bar{X} 2 mm)	4 mm	-	-
Neck	2-3 mm (\bar{X} 2.5 mm)	-	2 mm	-	-
Diameter of abdomen	3.5-5 mm (\bar{X} 4.2 mm)	1.8-2.2 mm (\bar{X} 2 mm)	4 mm	-	-
Hook parameter:			-	-	-
a. AC					
b. CB	0.23-0.55 mm	0.22-0.35 mm			
c. AD	0.37-0.69 mm 0.47-0.88 mm	0.29-0.35 mm 0.29-0.45 mm			
Mouth:			-	-	-
a. Length	0.16-29 mm	0.14-0.38 mm			
b. Width	0.12-0.24 mm	0.12-0.18 mm			
Egg:		-	-	-	
a. Length	114-130 μ m (\bar{X} 118 μ m)				125-154 μ m (\bar{X} 133 μ m)
b. Width	108-124 μ m (\bar{X} 116 μ m)				106-143 μ m (\bar{X} 123 μ m)

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138 **Figure 2.** Differences in size between female (top) and male (bellow).
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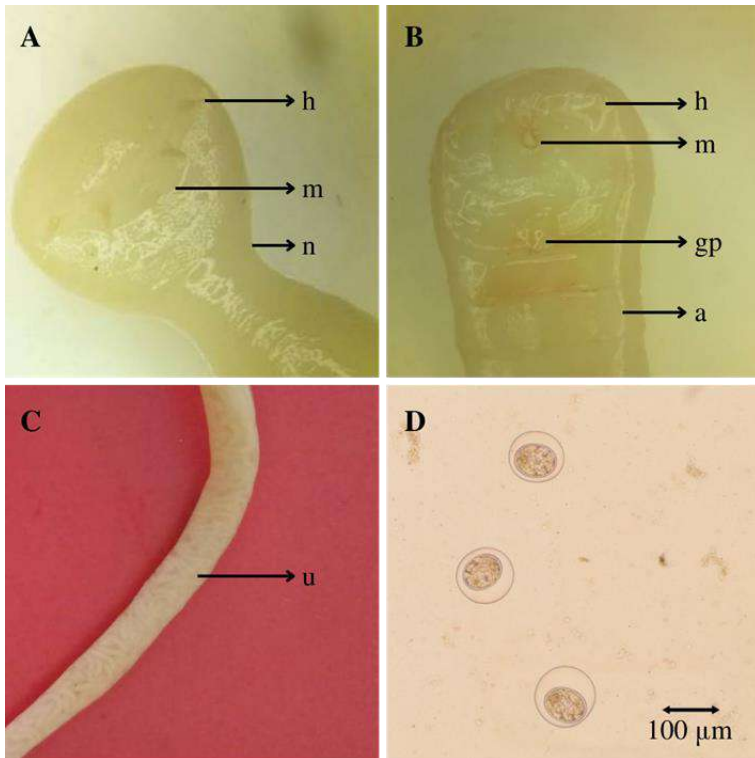
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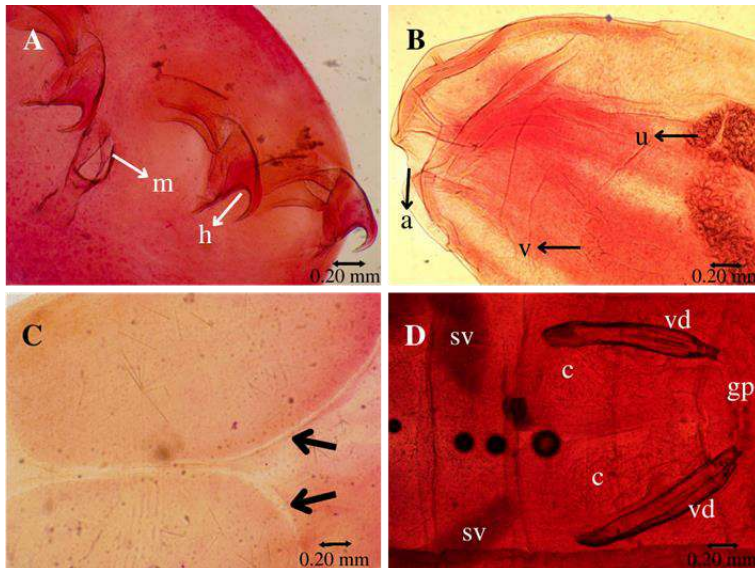
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Both sexes, adult males and female pentastomids, were found in this research (Figures 1 and 2). The number of male pentastomid was less (21 heads) than that of females (183 heads) Table 2. Table 2 also describes the size of each pentastomid. The female pentastomids had longer and larger bodies than the males (Figures 1 and 2). The length of the female bodies was 72-125 mm with an average of 94.5 mm. Their abdominal diameter was 3.5-5 mm with an average of 4.2 mm. Furthermore, the anterior section or cephalothorax of the females was globular with a neck separating their head and their abdomen (Figure 3A). The average length and width of the female mouths were 0.24 mm and 0.16 mm. Furthermore, the results of the microscopy examination using Carmine staining showed that the female pentastomid had two spermathecae on the anterior part that connect directly to the uterus that contains the egg (Figures 3C, 4B, and 4C). Vagina was in the posterior body and around the anus (Figure 4B).



150
151 **Figure 3.** Pentastomida without staining. (A) The anterior section of the female. (B) The anterior section of the male. (C) Female body.
152 (D) Pentastomid's eggs. h: hook, m: mouth, n: neck, gp: genital pore, a: first annuli, u: uterus.
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154 On the other hand, the length of the males' bodies was 17-28.5 mm with an average of 23.75 mm. Their abdominal
155 diameter was 1.8-2.2 mm with an average of 2 mm. The average length and width of the mouth were 0.23 and 0.14 mm,
156 respectively. The male body was simple and straight without any curves in the neck (Figure 3B). The genital pore was
157 identified in the first annulus and there was a pair of seminal vesicles, a pair of vasa deferens and cirrus (4D).
158



159
160 **Figure 4.** Carmine-stained pentastomida. (A) The anterior section. (B) The posterior section of the female. (C) A pair of female
161 spermatheca. (D) Male reproductive organs. m: mouth, h: hook, a: anus, v: vagina, u: uterus, sv: seminal vesicle, c: cirrus, vd: vas
162 deferens.
163

164 The eggs were oval and consisted of two layers of membrane that clearly could be distinguished. The first layer (outer
165 membrane) was thin, while the second layer (inner membrane) was thick and yellowish. There was fluid-filled space
166 between the first and second layers. The length of the eggs was 114-130 µm with an average of 118 µm and the width was
167 108-124 µm with an average of 116 µm (Figure 3D).

168 Pentastomida were mostly found in the lungs and the parasites also spread to the trachea in five snakes. This finding
169 was similar to research by Riley and Self (1980) that revealed that adult *Kiricephalus pattoni* (one of the pentastomida

170 species) were only found in the lungs of *P. mucosa* snakes. In our observation, pentastomids that spread to the trachea
171 were females. According to Sundar et al. (2015), the adult pentastomids with mature eggs are expelled from the trachea
172 and eliminated from the definitive host by oral expulsion or may also be swallowed, resulting in shed eggs in the feces in
173 which another intermediate mammal host will ingest to continue their life cycles.

174 The number of male pentastomids was fewer than the number of female ones. This case was also experienced by John
175 and Nadakal (1988) and Riley and Self (1980) that only found a few males compared the female. We agree with Almeida
176 et al. (2006) that pentastomids are not well known, in biology and the number of their species.

177 The taxonomy of pentastomida is still controversial. It has been known through molecular data that pentastomida are
178 closely related to crustaceans and belong to class Maxillopoda, sub-phylum Crustacea, and the phylum Arthropoda (Paré,
179 2008). Based on the size of the egg (Almeida et al., 2007), the form of the female head that is globular with a neck
180 separating the head from its body (Paré, 2008) and the size of the body length, the number of the annuli, the diameter of
181 cephalothorax, neck, abdominal diameter, head, and body shape (Riley and Self, 1979; Riley and Self, 1980), the
182 pentastomida found was classified as *K. pattoni*. *K. pattonii* can be classified into the order of porocephalida (Paré, 2008).
183 According to Christoffersen and De Assis (2013; 2015), pentastomid in *P. mucosa* snakes are classified into *K. pattoni*.

184 *K. pattoni* has a long annulated body, a tapered tail, an anal canal, two pairs of hooks or a total of four hooks on its
185 head, as well as a small ovoid-shaped mouth (Riley and Self, 1979). The female *K. pattoni* has a larger body than male one
186 (Paré, 2008). Female *K. pattoni* also has a spiral body with a larger head than its body, and a curve of the neck, whereas
187 male one has a straight body without a curve on the neck (Riley and Self, 1979; Riley and Self, 1980; John and Nadakal,
188 1988; Almeida et al., 2007). The spiral body in females is likely to facilitate their movement into the lung tissue of the
189 definitive hosts (John and Nadakal, 1988).

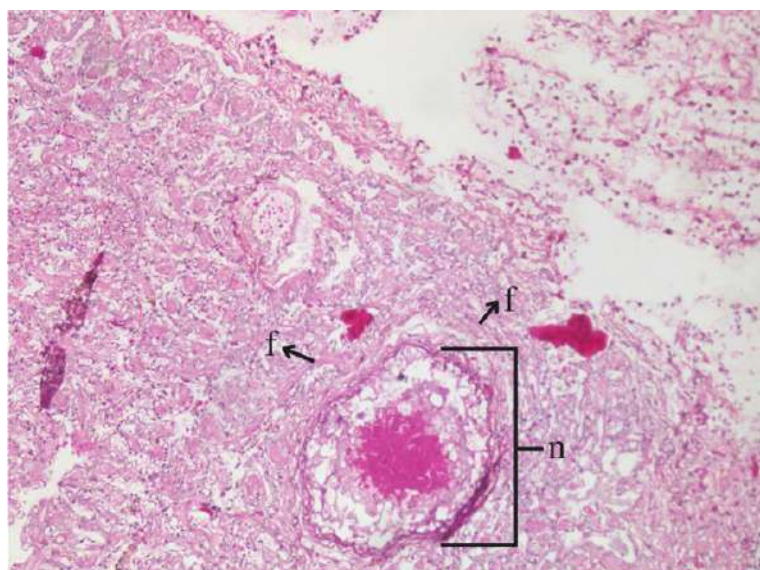
190 The morphology of the eggs like the eggs of *Porocephalus crotali* described by Sundar et al. (2015), the eggs were
191 composed of two-layer membranes of which the outer one is thin, and the inner membrane thick and there was a fluid-
192 filled space between two membranes. But the average diameter of the eggs measured in this investigation is smaller (118 x
193 116 µm) than the eggs of *P. crotali* (144 µm) as described by Sundar et al. (2015).

194

195 **Histopathological changes in the lungs of *Ptyas mucosa***

196 Squamates typically have two lungs, however, the left lung in most snakes is greatly reduced, never exceeding 85% of
197 the right lung's size, or absent. The right lung usually runs from near the heart to the cranial to the right kidney. The
198 cranial portion of a lung is vascularized and functions primarily as a gas exchanger (vascular lung), whereas the caudal
199 primarily functions as an air sac (saccular lung). The vascular lung's wall is made up of honeycombed units called faveoli,
200 which allow for gas exchange (Funk and Bogan, 2019). Microscopically, the faveoli in the snake lung parenchyma are
201 lined mainly by squamous epithelial cells (type I cells) and, to a lesser degree, by cuboidal epithelial cells (type II)
202 (Jacobson et al., 2021).

203 In this study, a nymphal stage of the parasite was found in the pulmonary tissue infected with pentastomida. The
204 parasite is surrounded by some connective tissue (Figure 5). Finding the stages of the nymph in the histological lung of the
205 snake and the stages of the parasite on the macroscopic lung organ means that the *P. mucosa* snake may act as an
206 intermediate and final host of *K. pattoni*. Within the lung cavities of the definitive host, the nymphs develop into adults
207 (Sundar et al., 2015). In this study, pentastomida infection in the lungs only caused a mild histological reaction in the form
208 of fibrosis in the area around the parasite.
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210 **Figure 5.** Histological microscopic picture of nymph-infected pulmo from pentastomida showed the presence of fibroblast around the
211 area of infection. f: fibroblast, n: the body of the nymph stage pentastomida.
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Pentastomida have an indirect life cycle that typically utilizes vertebrate hosts. Many of their species rely on reptiles as their definitive host, and the adults are usually found in the lungs (Wellehan and Walden, 2019). Pentastomida parasites migrate extensively through the visceral before settling in the lungs and, less typically, the trachea and nasal passageways. Pentastomida can produce localized lesions, whilst molting can elicit antigenic reactions. Adults feed on lung tissue fluids and pass embryonated eggs, which are coughed up through the oral cavity, swallowed, and passed in the feces (Comolli and Divers, 2021). In squamates, the pulmonary lumen is large, and the tracheal diameter may be smaller than that of the pentastomids. This poses a risk of respiratory obstruction by the adult pentastomes. However, the greatest pathological changes are seen in the intermediate host, where they may become embedded in tissues (Wellehan and Walden, 2019).

Pentastomiasis in reptiles, especially snakes, occurs subclinically, although the infection is large, and death is usually associated with severe pneumonia. Because pentastomids attach to the lung tissue, they can cause perforation, hemorrhaging, and serious inflammation in the host (Fernando and Fernando, 2014). In a Common Garter Snake (*Thamnophis sirtalis*), lung lesions were associated with one of the pentastomida species, *Kiricephalus coarctatus*, where the embedded parasite had gotten into the hypaxial muscle. Infections with *Armillifer armillatus* have also been linked to parasitic pneumonia in *Gaboon vipers*. Pentastomids occluding the trachea have been implicated in snake deaths. Bacterial infections could develop due to lung damage. Four of nine *Boa constrictors* with pulmonary damage from *Porocephalus dominicana* infection perished from bacteremia and pericarditis (Walden et al., 2021).

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CONCLUSION

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The prevalence of pentastomiasis in slaughtered *Ptyas mucosa* in Sidoarjo was 65% (39 of 60 snakes). The pentastomids that infect these snakes are classified as *Kiricephalus pattoni*. The pulmonary histological findings only show a light reaction in the form of mild fibrosis.

234

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1 First report of pentastomiasis and the lung histopathological changes in 2 *Ptyas*_{[JB1][A2]} *mucosa* in Sidoarjo, East Java, Indonesia

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12 **Abstract.** _{[This][JB3][A4]} study aimed to determine the prevalence of pentastomiasis, identify the parasite, and observe the pulmonary
13 histopathology in *Ptyas mucosa* collected from snake slaughter in Sidoarjo (Indonesia). _{[The][JB5][A6]} identification of pentastomids was
14 carried out by Semichen-Acetic Carmine, and histopathological preparations of the lungs were performed to observe the microscopic
15 lesions. The results showed that 65% of the snakes had pentastomiasis, and the parasites were characterized as *Kirichephalus pattoni*.
16 Microscopical examination revealed mild fibrosis in the pulmonary area as a result of pentastomid infestation. This is the first report of
17 some pentastomiasis in *P. mucosa* in Sidoarjo, East Java, _{[Indonesia][JB7][A8]}.

18 **Key words:** *Kirichephalus pattoni*, Parasitic Pneumonia, Rat Snake, _{[Zoonosis][JB9][A10]}.

19 **Abbreviations:**

20 **Running title:** Pentastomiasis in Rat Snake

21 INTRODUCTION

22 Exotic animals such as reptiles, especially various types of snakes, are now popular as pets throughout the world
23 (Okulewicz et al., 2014). However, they are also concerned with the increasing animal welfare and health issues through
24 the animal trade in the market (Yudhana et al., 2021). Some species of snake are bred in captivity, while others are caught
25 in the wild, and sold as pets or as bushmeat for consumption _(Hardi et al., 2017; Yudhana et al., 2021). _{[JB11][A12]}

26 *Ptyas mucosa* is one of the most common snake species found in _{[Indonesia][JB13][A14]}. This type of snake, known as the
27 Oriental rat snake and Indian rat snake, belongs to the Colubridae family which is widely distributed throughout East,
28 South, and Southeast Asia (Auliya, 2010; Muliya and Bhat, 2016; Yuan et al., 2022). *P. mucosa* is a diurnal and semi-
29 arboreal snake species that lives on forest floors, agricultural fields, wet ground, and in close proximity to human habitats.
30 It feeds on frogs, rats, and other small animals (Chittora et al., 2021).

31 The meat of *P. mucosa* is traded legally in _{[China][JB15][A16]}. The meat and internal organs are used as fresh or frozen
32 food, the bile and blood are used in traditional medicine, and the skin is exported to several European countries as raw
33 materials for the leather _{[industry][JB17][A18]}. Although it is illegal, most rural communities in Indonesia trade and consume
34 the meat and bile of *P. mucosa* for some health reasons _(Auliya, 2010)[JB19][A20]; Pranashinta et al., 2017).

35 Wildlife meat, however, poses a threat of transmitting infection with novel and exotic pathogens to human hosts (Hardi
36 et al., 2017). Then new zoonotic diseases are likely to emerge consequently. For instance, Bats (Chiroptera) are important
37 reservoirs of several emerging diseases, including Ebola virus disease (EVD), severe acute respiratory syndrome (SARS),
38 and Middle East respiratory syndrome (MERS), as well as the current pandemic COVID-19 caused by SARS Coronavirus
39 2 (Irving et al., 2021). Many types of parasites are also the cause of zoonotic emerging diseases, resulting in a significant
40 public health burden, particularly in tropical countries (Alasil and Abdullah, 2019).

41 Several parasites found in confined reptiles spend a portion of their life cycle in the respiratory system. The majority of
42 respiratory involvement is _{[asymptomatic and modest][JB21][A22]}, generating mostly localized inflammation, with the
43 exception of instances with excessive parasite loads. However, severe disease can result from high burdens or unusual
44 host-parasite interactions, which is frequently made worse by secondary infections (Comolli, 2021). Reptile-borne
45 pentastomiasis is an unusual tropical disease that is neglected and under-researched in Asia, Africa, and South America
46 (Sulyok et al., 2014).

47 Pentastomiasis is a parasitic zoonosis disease [JB23][A24] caused by infection with pentastomids, belonging to the
48 subclass Pentastomida, under the phylum Arthropoda (Chittora et al., 2021; Tongtako et al., 2013; WHA, 2019).
49 Pentastomida are also known as tongue worms since the adult phase of the genus [Linguatula][JB25][A26] has similarities
50 with the mammalian tongues (Paré, 2008). Adult parasites are usually found in the respiratory tract of reptiles, birds, and
51 mammals, including humans, while their larval forms are in the internal organs of insects, mammals, and amphibians
52 (Paré, 2008; Chen et al., 2010; Kelehear et al., 2014). Some zoonotic pentastomida in snakes are Armillifer and
53 *Porocephalus crotali* (Rataj et al., 2011). Humans are infected with pentastomids since they consume raw snake meat or
54 drink water contaminated with parasitic eggs (Ayinmode et al., 2010; Kelehear et al., 2014; Tappe et al., 2016).

55 Pentastomiasis in snakes have been reported in many countries, such as poisonous *Bothrops asper* in Costa Rica
56 (Alvarado et al., 2015), Royal Python [Python][JB27][A28] snakes in Nigeria (Ayinmode et al., 2010), Rat snakes in Sri Lanka and
57 India (Fernando and Fernando, 2014; Sundar et al., 2015), wild snakes in Australia (Kelehear et al., 2014), various species
58 of snakes in Slovenia and Pakistan (Rataj et al., 2011), *Agkistrodon acutus* and *Python molurus* in China (Chen et al.,
59 2010), and *Crotalus totonacus* in Mexico [JB29][A30] (Tepos-Ramírez et al., 2022).

60 To the best of our knowledge, there are no reports of pentastomids infections in snakes in Indonesia. Therefore, this
61 study reports the prevalence of pentastomiasis, the identification of pentastomids, and the pulmonary histopathological
62 profiles in Rat snakes (*P. mucosa*) in Sidoarjo Sub-district, East Java, Indonesia.

63

MATERIALS AND METHODS

64 Ethical approval

65 This study was reviewed and approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine,
66 Universitas Airlangga (number 678-KE).

67

68 Parasites collection [JB31][A32]

69 The collection was conducted at a snake slaughterhouse in Tulangan District, Sidoarjo Regency, East Java Province,
70 Indonesia. The presence of pentastomid parasites was observed in the respiratory tract of sixty slaughtered *Ptyas mucosa*
71 [JB33][A34] snakes. Pentastomides were taken and cleaned with distilled water, then stored in sterile plastic pots. The lungs
72 of the P [JB35][A36]. *mucosa* infected with the parasite were also isolated for histopathological preparation [JB37][A38][A39].

73

74 Prevalence calculation

75 The prevalence of pentastomiasis in snake samples was calculated by the following formula:

76
$$\text{Prevalence (\%)} = (\text{The number of snakes infected with pentastomids}) / (\text{Total sample of snakes}) \times 100\%.$$

77

78 Semichen-acetic carmine staining

79 The collected pentastomids were stained with the Semichen-Acetic Carmine technique [JB40] (Kuhlmann, 2006). Adult
80 pentastomids were fixed on object glasses and soaked in 5% glycerin alcohol for 24 hours. Then they were soaked in 70%
81 alcohol for five minutes. Subsequently, the object glasses with pentastomids were placed in Carmine alcohol solution (1:2)
82 for eight hours. The pentastomids were soaked in acid alcohol for 10 minutes and moved to base alcohol for 20 minutes.
83 Furthermore, graded dehydration was carried out with 70%, 85% and 95% alcohol for five minutes, respectively. The
84 mounting step was done using Hung's I for 20 minutes. Pentastomids were taken and placed on new clean object glasses.
85 The pentastomids were dripped with Hung's II solution and then covered with a cover glass.

86

87 Parasite identification

88 Characteristics of the head and body shape and body size of the parasite were identified based on Riley and Self
89 (1980), Almedia et al. (2006), and Christoffersen and De Assis (2013; 2015). Identification of parasite egg size was carried
90 out according to Keegan [JB41][A42] (1943).

91 The length of the fresh parasites was measured from the anterior edge to the posterior end. The head was observed
92 under a stereo microscope and documented with a Canon Digital Single-Lens Reflex (DSLR) camera. Stained parasites
93 were observed using a light microscope with 40-100× magnification and documented using Miconos Optilab. Some
94 female parasites were incubated in PBS overnight at 37 °C to collect the eggs. The supernatants were centrifuged at 1,500
95 rpm for 10 minutes and the pellet was dripped onto a glass object and observed using a light microscope with 100×
96 magnification. Eggs were measured using Miconos Optilab.

97

98 Histopathological preparations

99 The lungs of snakes with pentastomid infestation were fixed in 10% formalin. Then graded dehydration was carried out
100 with serial alcohol concentrations (70%, 80%, 90%, 96%, and absolute) and clearing using xylol. Furthermore, paraffin
101 embedding was performed to obtain a solid block of tissue. The tissue blocks were sliced (3 micrometers thick) using a
102 microtome, mounted on an object glass, and stained using Hematoxylin and Eosin. Microscopic examination was
103 performed using Nikon Eclipse Ci.

105 The prevalence of pentastomiasis in *Ptyas mucosa* snakes

106 Thirty-nine or 65% of the respiratory tract of 60 were positively infected with adult pentastomids. [Totally][JB43][A44],
 107 204 pentastomids were collected [JB45][A46] (Table 1). Pentastomida were found to be strongly attached to the trachea and
 108 lungs of snakes (Figure 1). Some pentastomids even buried their heads in the lungs of snakes. The parasites of all snakes
 109 positively infected with pentastomids were found in the lungs (39 snakes), while only 5 snakes which parasites spread to
 110 their tracheas.

111

112 **Table 1.** Prevalence of pentastomiasis in *Ptyas mucosa* based on site and number of parasites.

Sites	Positive (%)	Number of parasites
Lungs [JB47][A48]	57% (34 of 60)	2-9 with the average of 5 pentastomida
Trachea	0% (0 of 60)	-
Lungs and Trachea	8% (5 of 60)	7-9 with the average of 8 pentastomida
Total	65% (39 of 60)	204 pentastomida

113



114

115 **Figure 1.** Pentastomid infestation in the lungs of *Ptyas mucosa*. The white arrows show areas with raised lesions around the attachment
 116 of pentastomids to the tissue. The black arrows show female pentastomids and the red arrow shows male pentastomids.

117

118 This is the first study to determine the prevalence of pentastomiasis in *Ptyas mucosa* in Sidoarjo, East Java Indonesia.
 119 As shown in our results, pentastomids were present in 39 of 60 (65%) snakes. Based on this finding, the prevalence of
 120 pentastomiasis in *P. mucosa* in this study was quite high. In this case, it was slightly the same as the prevalence
 121 of pentastomiasis in Brazil, 8 of 15 or 53.33% of snakes were positively infected with pentastomid. The high prevalence in
 122 this research was probably caused by the food consumed by the snakes. The type of food is very influential on the
 123 prevalence of infection and the type of pentastomida that infected the snakes (Almeida et al., 2007). *P. mucosa* snakes
 124 usually feed on rats, frogs, toads, lizards, and even smaller non-venomous and smaller snakes (Sidik, 2006). According to
 125 Esslinger (1962), rats, frogs, toads, lizards, and even non-venomous snakes can act as the intermediate hosts of
 126 pentastomida.

127

128 Identification of pentastomida

129 The characteristics of pentastomid found in this study can be seen in Table 2 and Figure 2. Pentastomids have a long
 130 cylindrical body with a larger head, a flat shape on the ventral, and a tapered tail or posterior. Their body was soft and
 131 whitish with a colorless cuticle, which tends to be transparent so that the internal organs are visible. There were annuli
 132 throughout their body up to the posterior. There are two pairs of hooks (a total of four hooks) around the head that are used
 133 to attach to the host's lung tissue, and it had an ovoid-shaped mouth between the two pairs of hooks [JB51][A52].

134

135 **Table 2.** The characteristics of pentastomida compared with *Kiricephalus pattoni* by Riley and Self (1980) and Keegan
 136 (1943).

Parameters	The current study		British Museum collection (Riley and Self, 1980)		Keegan, 1943
	Females	Males	Females	Males	
Total number of parasites	183	21	17	2	-
Body length	72-125 mm (\bar{X} 94.5 mm)	17-28.5 mm (\bar{X} 23.75 mm)	83-137 mm (\bar{X} 100 mm)	28-29 mm (\bar{X} 28.5 mm)	-
Number of	29-41	28-35	34-38	31-32	-

<i>annuli</i>	(\bar{X} 31.6)	(\bar{X} 31)	(\bar{X} 36.5)	(\bar{X} 31.5)	
Diameter of cephalothorax	4-5.5 mm (\bar{X} 4.75 mm)	2-2.5 mm (\bar{X} 2 mm)	4 mm	-	-
Neck	2-3 mm (\bar{X} 2.5 mm)	-	2 mm	-	-
Diameter of abdomen	3.5-5 mm (\bar{X} 4.2 mm)	1.8-2.2 mm (\bar{X} 2 mm)	4 mm	-	-
Hook parameter:			-	-	-
a. AC					
b. CB	0.23-0.55 mm	0.22-0.35 mm			
c. AD	0.37-0.69 mm 0.47-0.88 mm	0.29-0.35 mm 0.29-0.45 mm			
Mouth:			-	-	-
a. Length	0.16-29 mm	0.14-0.38 mm			
b. Width	0.12-0.24 mm	0.12-0.18 mm			
Egg:		-	-	-	
a. Length	114-130 μ m (\bar{X} 118 μ m)				125-154 μ m (\bar{X} 133 μ m)
b. Width	108-124 μ m (\bar{X} 116 μ m)				106-143 μ m (\bar{X} 123 μ m)

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Figure 2. Differences in size between female (top) and male (bellow).

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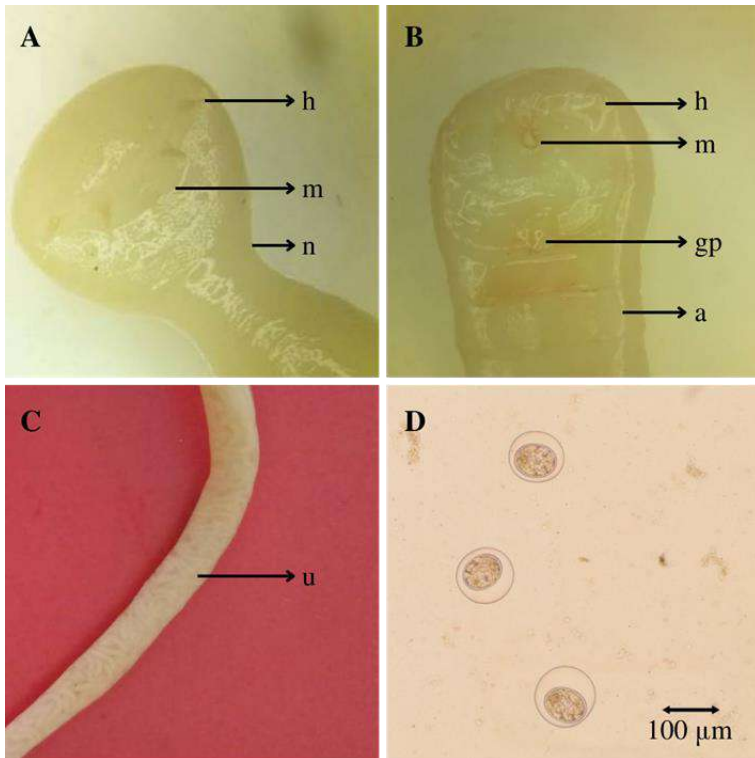
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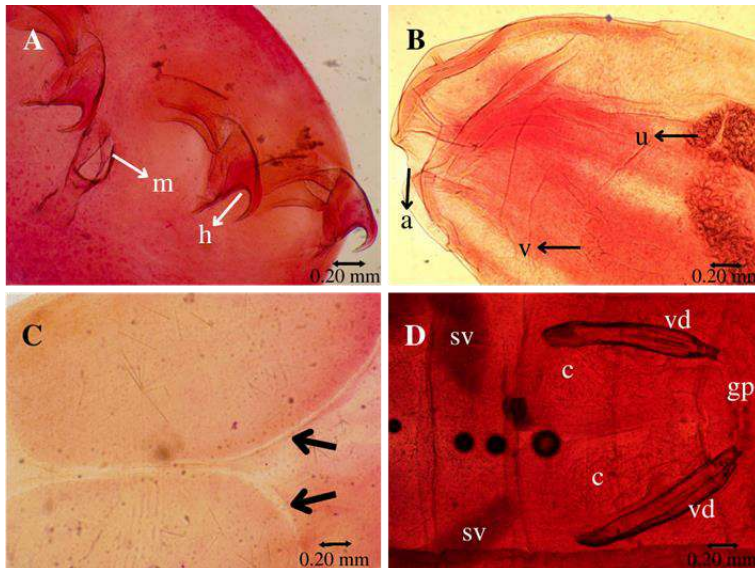
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Both sexes, adult males and female pentastomids, were found in this research (Figures 1 and 2). The number of male pentastomid was less (21 heads) than that of females (183 heads) Table 2. Table 2 also describes the size of each pentastomid. The female pentastomids had longer and larger bodies than the males (Figures 1 and 2). The length of the female bodies was 72-125 mm with an average of 94.5 mm. Their abdominal diameter was 3.5-5 mm with an average of 4.2 mm. Furthermore, the anterior section or cephalothorax of the females was globular with a neck separating their head and their abdomen (Figure 3A). The average length and width of the female mouths were 0.24 mm and 0.16 mm. Furthermore, the results of the microscopy examination using Carmine staining showed that the female pentastomid had two spermathecae on the anterior part that connect directly to the uterus that contains the egg (Figures 3C, 4B, and 4C). Vagina was in the posterior body and around the anus (Figure 4B).



151
152 **Figure 3.** Pentastomida without staining. (A) The anterior section of the female. (B) The anterior section of the male. (C) Female body.
153 (D) Pentastomid's eggs. h: hook, m: mouth, n: neck, gp: genital pore, a: first annuli, u: uterus.
154

155 On the other hand, the length of the males' bodies was 17-28.5 mm with an average of 23.75 mm. Their abdominal
156 diameter was 1.8-2.2 mm with an average of 2 mm. The average length and width of the mouth were 0.23 and 0.14 mm,
157 respectively. The male body was simple and straight without any curves in the neck (Figure 3B). The genital pore was
158 identified in the first annulus and there was a pair of seminal vesicles, a pair of vasa deferens and cirrus (4D).
159



160
161 **Figure 4.** Carmine-stained pentastomida. (A) The anterior section. (B) The posterior section of the female. (C) A pair of female
162 spermatheca. (D) Male reproductive organs. m: mouth, h: hook, a: anus, v: vagina, u: uterus, sv: seminal vesicle, c: cirrus, vd: vas
163 deferens.
164

165 The eggs were oval and consisted of two layers of membrane that clearly could be distinguished. The first layer (outer
166 membrane) was thin, while the second layer (inner membrane) was thick and yellowish. There was fluid-filled space
167 between the first and second layers. The length of the eggs was 114-130 µm with an average of 118 µm and the width was
168 108-124 µm with an average of 116 µm (Figure 3D).

169 Pentastomida were mostly found in the lungs and the parasites also spread to the trachea in five snakes. This finding
170 was similar to research by Riley and Self (1980) that revealed that adult *Kiricephalus pattoni* (one of the pentastomida

171 species) were only found in the lungs of *P. mucosa* snakes. In our observation, pentastomids that spread to the trachea
172 were females. According to Sundar et al. (2015), the adult pentastomids with mature eggs are expelled from the trachea
173 and eliminated from the definitive host by oral expulsion or may also be swallowed, resulting in shed eggs in the feces in
174 which another intermediate mammal host will ingest to continue their life cycles.

175 The number of male pentastomids was fewer than the number of female ones. This case was also experienced by John
176 and Nadakal (1988) and Riley and Self (1980) that only found a few males compared the female. We agree with Almeida
177 et al. (2006) that pentastomids are not well known, in biology and the number of their species.

178 The taxonomy of pentastomida is still controversial. It has been known through molecular data that pentastomida are
179 closely related to crustaceans and belong to class Maxillopoda, sub-phylum Crustacea, and the phylum Arthropoda (Paré,
180 2008). Based on the size of the egg (Almeida et al., 2007), the form of the female head that is globular with a neck
181 separating the head from its body (Paré, 2008) and the size of the body length, the number of the annuli, the diameter of
182 cephalothorax, neck, abdominal diameter, head, and body shape (Riley and Self, 1979; Riley and Self, 1980), the
183 pentastomida found was classified as *K. pattoni*. *K. pattonii* can be classified into the order of porocephalida (Paré, 2008).
184 According to Christoffersen and De Assis (2013; 2015), pentastomid in *P. mucosa* snakes are classified into *K. pattoni*.

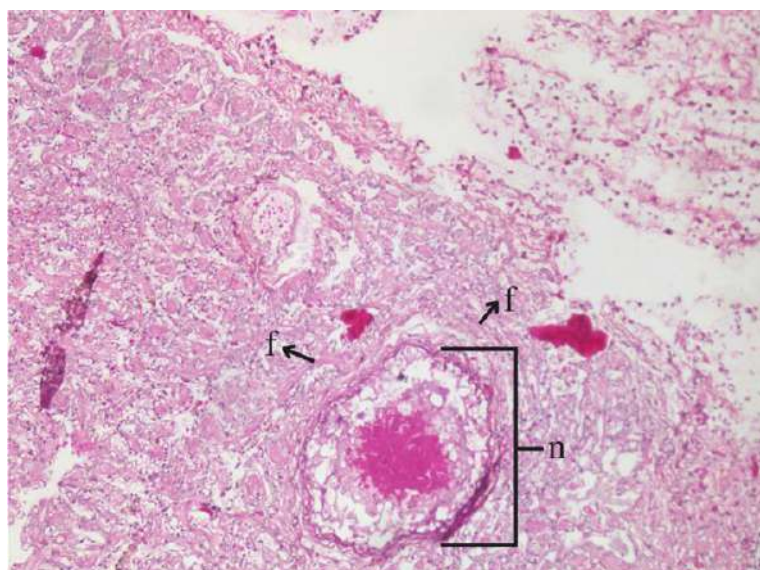
185 *K. pattoni* has a long annulated body, a tapered tail, an anal canal, two pairs of hooks or a total of four hooks on its
186 head, as well as a small ovoid-shaped mouth (Riley and Self, 1979). The female *K. pattoni* has a larger body than male one
187 (Paré, 2008). Female *K. pattoni* also has a spiral body with a larger head than its body, and a curve of the neck, whereas
188 male one has a straight body without a curve on the neck (Riley and Self, 1979; Riley and Self, 1980; John and Nadakal,
189 1988; Almeida et al., 2007). The spiral body in females is likely to facilitate their movement into the lung tissue of the
190 definitive hosts (John and Nadakal, 1988).

191 The morphology of the eggs like the eggs of *Porocephalus crotali* described by Sundar et al. (2015), the eggs were
192 composed of two-layer membranes of which the outer one is thin, and the inner membrane thick and there was a fluid-
193 filled space between two membranes. But the average diameter of the eggs measured in this investigation is smaller (118 x
194 116 µm) than the eggs of *P. crotali* (144 µm) as described by Sundar et al. (2015).

195 **Histopathological changes in the lungs of *Ptyas mucosa***

196 Squamates typically have two lungs, however, the left lung in most snakes is greatly reduced, never exceeding 85% of
197 the right lung's size, or absent [JB53] [A54]. The right lung usually runs from near the heart to the cranial to the right
198 kidn [JB55] [A56]ey. The cranial portion of a lung is vascularized and functions primarily as a gas exchanger (vascular lung),
199 whereas the caudal primarily functions as an air sac (saccular lung). The vascular lung's wall is made up of honeycombed
200 units called faveoli, which allow for gas exchange (Funk and Bogan, 2019). Microscopically, the faveoli in the snake lung
201 parenchyma are lined mainly by squamous epithelial cells (type I cells) and, to a lesser degree, by cuboidal epithelial cells
202 (type II) (Jacobson et al., 2021).

203 In this study, a nymphal stage of the parasite was found in the pulmonary tissue infected with pentastomida. The
204 parasite is surrounded by some connective tissue (Figure 5). Finding the stages of the nymph in the histological lung of the
205 snake and the stages of the parasite on the macroscopic lung organ means that the *P. mucosa* snake may act as an
206 intermediate and final host of *K. pattoni*. Within the lung cavities of the definitive host, the nymphs develop into adults
207 (Sundar et al., 2015). In this study, pentastomida infection in the lungs only caused a mild histological reaction in the form
208 of fibrosis in the area around the parasite.
209
210



211 **Figure 5.** Histological microscopic picture of nymph-infected pulmo from pentastomida showed the presence of fibroblast around the
212 area of infection. f: fibroblast, n: the body of the nymph stage pentastomida.
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Pentastomida have an indirect life cycle that typically utilizes vertebrate hosts. Many of their species rely on reptiles as their definitive host, and the adults are usually found in the lungs (Wellehan and Walden, 2019). Pentastomida parasites migrate extensively through the visceral before settling in the lungs and, less typically, the trachea and nasal passageways. Pentastomida can produce localized lesions, whilst molting can elicit antigenic reactions. Adults feed on lung tissue fluids and pass embryonated eggs, which are coughed up through the oral cavity, swallowed, and passed in the feces (Comolli and Divers, 2021). In squamates, the pulmonary lumen is large, and the tracheal diameter may be smaller than that of the pentastomids. This poses a risk of respiratory obstruction by the adult pentastomes. However, the greatest pathological changes are seen in the intermediate host, where they may become embedded in tissues (Wellehan and Walden, 2019[JB57][A58]).

Pentastomiasis in reptiles, especially snakes, occurs subclinically, although the infection is large, and death is usually associated with severe pneumonia[JB59][A60]. Because pentastomids attach to the lung tissue, they can cause perforation, hemorrhaging, and serious inflammation in the host (Fernando and Fernando, 2014). In a Common Garter Snake (*Thamnophis sirtalis*), lung lesions were associated with one of the pentastomida species, *Kiricephalus coarctatus*, where the embedded parasite had gotten into the hypaxial muscle. Infections with *Armillifer armillatus* have also been linked to parasitic pneumonia in *Gaboon vipers*. Pentastomids occluding the trachea have been implicated in snake deaths. Bacterial infections could develop due to lung damage. Four of nine *Boa constrictors* with pulmonary damage from *Porocephalus dominicana* infection perished from bacteremia and pericarditis (Walden et al., 2021).

232

CONCLUSION

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The prevalence of pentastomiasis in slaughtered *Ptyas mucosa* in Sidoarjo was 65% (39 of 60 snakes). The pentastomids that infect these snakes are classified as *Kiricephalus pattoni*. The pulmonary histological findings only show a light reaction in the form of mild fibrosis.

236

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237

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1 First report of pentastomiasis and the lung histopathological changes in 2 *Ptyas*^[JB1] *mucosa* in Sidoarjo, East Java, Indonesia

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12 **Abstract.** ^[This]^[JB2] study aimed to determine the prevalence of pentastomiasis, identify the parasite, and observe the pulmonary
13 histopathology in *Ptyas mucosa* collected from snake slaughter in Sidoarjo (Indonesia). ^[The]^[JB3] identification of pentastomids was
14 carried out by Semichen-Acetic Carmine, and histopathological preparations of the lungs were performed to observe the microscopic
15 lesions. The results showed that 65% of the snakes had pentastomiasis, and the parasites were characterized as *Kirichephalus pattoni*.
16 Microscopical examination revealed mild fibrosis in the pulmonary area as a result of pentastomid infestation. This is the first report of
17 some pentastomiasis in *P. mucosa* in Sidoarjo, East Java, ^[Indonesia]^[JB4].

18 **Key words:** *Kirichephalus pattoni*, Parasitic Pneumonia, Rat Snake, ^[Zoonosis]^[JB5].

19 **Abbreviations:**

20 **Running title:** Pentastomiasis in Rat Snake

21 INTRODUCTION

22 Exotic animals such as reptiles, especially various types of snakes, are now popular as pets throughout the world
23 (Okulewicz et al., 2014). However, they are also concerned with the increasing animal welfare and health issues through
24 the animal trade in the market (Yudhana et al., 2021). Some species of snake are bred in captivity, while others are caught
25 in the wild, and sold as pets or as bushmeat for consumption (^[Hardi et al., 2017; Yudhana et al., 2021]^[JB6]).

26 *Ptyas mucosa* is one of the most common snake species found in ^[Indonesia]^[JB7]. This type of snake, known as the
27 Oriental rat snake and Indian rat snake, belongs to the Colubridae family which is widely distributed throughout East,
28 South, and Southeast Asia (Auliya, 2010; Muliya and Bhat, 2016; Yuan et al., 2022). *P. mucosa* is a diurnal and semi-
29 arboreal snake species that lives on forest floors, agricultural fields, wet ground, and in close proximity to human habitats.
30 It feeds on frogs, rats, and other small animals (Chittora et al., 2021).

31 The meat of *P. mucosa* is traded legally in ^[China]^[JB8]. The meat and internal organs are used as fresh or frozen food,
32 the bile and blood are used in traditional medicine, and the skin is exported to several European countries as raw materials
33 for the leather ^[industry]^[JB9]. Although it is illegal, most rural communities in Indonesia trade and consume the meat and
34 bile of *P. mucosa* for some health reasons (^[Auliya, 2010]^[JB10]; Pranashinta et al., 2017).

35 Wildlife meat, however, poses a threat of transmitting infection with novel and exotic pathogens to human hosts (Hardi
36 et al., 2017). Then new zoonotic diseases are likely to emerge consequently. For instance, Bats (Chiroptera) are important
37 reservoirs of several emerging diseases, including Ebola virus disease (EVD), severe acute respiratory syndrome (SARS),
38 and Middle East respiratory syndrome (MERS), as well as the current pandemic COVID-19 caused by SARS Coronavirus
39 2 (Irving et al., 2021). Many types of parasites are also the cause of zoonotic emerging diseases, resulting in a significant
40 public health burden, particularly in tropical countries (Alasil and Abdullah, 2019).

41 Several parasites found in confined reptiles spend a portion of their life cycle in the respiratory system. The majority of
42 respiratory involvement is ^[asymptomatic and modest]^[JB11], generating mostly localized inflammation, with the exception
43 of instances with excessive parasite loads. However, severe disease can result from high burdens or unusual host-parasite
44 interactions, which is frequently made worse by secondary infections (Comolli, 2021). Reptile-borne pentastomiasis is an
45 unusual tropical disease that is neglected and under-researched in Asia, Africa, and South America (Sulyok et al., 2014).

46 Pentastomiasis is a parasitic zoonosis disease [JB12] caused by infection with pentastomids, belonging to the subclass
47 Pentastomida, under the phylum Arthropoda (Chittora et al., 2021; Tongtako et al., 2013; WHA, 2019). Pentastomida are
48 also known as tongue worms since the adult phase of the genus *Linguatula* [JB13] has similarities with the mammalian
49 tongues (Paré, 2008). Adult parasites are usually found in the respiratory tract of reptiles, birds, and mammals, including
50 humans, while their larval forms are in the internal organs of insects, mammals, and amphibians (Paré, 2008; Chen et al.,
51 2010; Kelehear et al., 2014). Some zoonotic pentastomida in snakes are *Armillifer* and *Porocephalus crotali* (Rataj et al.,
52 2011). Humans are infected with pentastomids since they consume raw snake meat or drink water contaminated with
53 parasitic eggs (Ayinmode et al., 2010; Kelehear et al., 2014; Tappe et al., 2016).

54 Pentastomiasis in snakes have been reported in many countries, such as poisonous *Bothrops asper* in Costa Rica
55 (Alvarado et al., 2015), Royal Python [JB14] snakes in Nigeria (Ayinmode et al., 2010), Rat snakes in Sri Lanka and India
56 (Fernando and Fernando, 2014; Sundar et al., 2015), wild snakes in Australia (Kelehear et al., 2014), various species of
57 snakes in Slovenia and Pakistan (Rataj et al., 2011), *Agkistrodon acutus* and *Python molurus* in China (Chen et al., 2010),
58 and *Crotalus totonacus* in Mexico [JB15] (Tepos-Ramírez et al., 2022).

59 To the best of our knowledge, there are no reports of pentastomids infections in snakes in Indonesia. Therefore, this
60 study reports the prevalence of pentastomiasis, the identification of pentastomids, and the pulmonary histopathological
61 profiles in Rat snakes (*P. mucosa*) in Sidoarjo Sub-district, East Java, Indonesia.

62 MATERIALS AND METHODS

63 Ethical approval

64 This study was reviewed and approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine,
65 Universitas Airlangga (number 678-KE).

66 Parasites collection [JB16]

67 The collection was conducted at a snake slaughterhouse in Tulangan District, Sidoarjo Regency, East Java Province,
68 Indonesia. The presence of pentastomid parasites was observed in the respiratory tract of sixty slaughtered *Ptyas mucosa*
69 [JB17] snakes. Pentastomides were taken and cleaned with distilled water, then stored in sterile plastic pots. The lungs of the
70 P [JB18]. *mucosa* infected with the parasite were also isolated for histopathological preparation [JB19].

71 Prevalence calculation

72 The prevalence of pentastomiasis in snake samples was calculated by the following formula:

73
$$\text{Prevalence (\%)} = (\text{The number of snakes infected with pentastomids}) / (\text{Total sample of snakes}) \times 100\%.$$

74 Semichen-acetic carmine staining

75 The collected pentastomids were stained with the Semichen-Acetic Carmine technique [JB20] (Kuhlmann, 2006). Adult
76 pentastomids were fixed on object glasses and soaked in 5% glycerin alcohol for 24 hours. Then they were soaked in 70%
77 alcohol for five minutes. Subsequently, the object glasses with pentastomids were placed in Carmine alcohol solution (1:2)
78 for eight hours. The pentastomids were soaked in acid alcohol for 10 minutes and moved to base alcohol for 20 minutes.
79 Furthermore, graded dehydration was carried out with 70%, 85% and 95% alcohol for five minutes, respectively. The
80 mounting step was done using Hung's I for 20 minutes. Pentastomids were taken and placed on new clean object glasses.
81 The pentastomids were dripped with Hung's II solution and then covered with a cover glass.

82 Parasite identification

83 Characteristics of the head and body shape and body size of the parasite were identified based on Riley and Self
84 (1980), Almedia et al. (2006), and Christoffersen and De Assis (2013; 2015). Identification of parasite egg size was carried
85 out according to Keegan [JB21] (1943).

86 The length of the fresh parasites was measured from the anterior edge to the posterior end. The head was observed
87 under a stereo microscope and documented with a Canon Digital Single-Lens Reflex (DSLR) camera. Stained parasites
88 were observed using a light microscope with 40-100× magnification and documented using Miconos Optilab. Some
89 female parasites were incubated in PBS overnight at 37 °C to collect the eggs. The supernatants were centrifuged at 1,500
90 rpm for 10 minutes and the pellet was dripped onto a glass object and observed using a light microscope with 100×
91 magnification. Eggs were measured using Miconos Optilab.

92 Histopathological preparations

93 The lungs of snakes with pentastomid infestation were fixed in 10% formalin. Then graded dehydration was carried out
94 with serial alcohol concentrations (70%, 80%, 90%, 96%, and absolute) and clearing using xylol. Furthermore, paraffin
95 embedding was performed to obtain a solid block of tissue. The tissue blocks were sliced (3 micrometers thick) using a
96 microtome, mounted on an object glass, and stained using Hematoxylin and Eosin. Microscopic examination was
97 performed using Nikon Eclipse Ci.

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RESULTS AND DISCUSSIONS

The prevalence of pentastomiasis in *Ptyas mucosa* snakes

Thirty-nine or 65% of the respiratory tract of 60 were positively infected with adult pentastomids. [Totally][JB22], 204 pentastomids were collected[JB23] (Table 1). Pentastomida were found to be strongly attached to the trachea and lungs of snakes (Figure 1). Some pentastomids even buried their heads in the lungs of snakes. The parasites of all snakes positively infected with pentastomids were found in the lungs (39 snakes), while only 5 snakes which parasites spread to their tracheas.

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Table 1. Prevalence of pentastomiasis in *Ptyas mucosa* based on site and number of parasites.

Sites	Positive (%)	Number of parasites
Lungs[JB24]	57% (34 of 60)	2-9 with the average of 5 pentastomida
Trachea	0% (0 of 60)	-
Lungs and Trachea	8% (5 of 60)	7-9 with the average of 8 pentastomida
Total	65% (39 of 60)	204 pentastomida

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Figure 1. Pentastomid infestation in the lungs of *Ptyas mucosa*. The white arrows show areas with raised lesions around the attachment of pentastomids to the tissue. The black arrows show female pentastomids and the red arrow shows male pentastomids.

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This is the first study to determine the prevalence of pentastomiasis in *Ptyas mucosa* in Sidoarjo, East Java Indonesia. As shown in our results, pentastomids were present in 39 of 60 (65%) snakes. Based on this finding, the prevalence of pentastomiasis in *P. mucosa* in this study was quite high. In this case, it was slightly the same as the prevalence of pentastomiasis in Brazil, 8 of 15 or 53.33% of snakes were positively infected with pentastomid. The high prevalence in this research was probably caused by the food consumed by the snakes. The type of food is very influential on the prevalence of infection and the type of pentastomida that infected the snakes (Almeida et al., 2007). *P. mucosa* snakes usually feed on rats, frogs, toads, lizards, and even smaller non-venomous and smaller snakes (Sidik, 2006). According to Esslinger (1962), rats, frogs, toads, lizards, and even non-venomous snakes can act as the intermediate hosts of pentastomida.

Identification of pentastomida

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The characteristics of pentastomid found in this study can be seen in Table 2 and Figure 2. Pentastomids have a long cylindrical body with a larger head, a flat shape on the ventral, and a tapered tail or posterior. Their body was soft and whitish with a colorless cuticle, which tends to be transparent so that the internal organs are visible. There were annuli throughout their body up to the posterior. There are two pairs of hooks (a total of four hooks) around the head that are used to attach to the host's lung tissue, and it had an ovoid-shaped mouth between the two pairs of hooks[JB26].

Table 2. The characteristics of pentastomida compared with *Kiricephalus pattoni* by Riley and Self (1980) and Keegan (1943).

Parameters	The current study		British Museum collection (Riley and Self, 1980)		Keegan, 1943
	Females	Males	Females	Males	
Total number of parasites	183	21	17	2	-
Body length	72-125 mm (\bar{X} 94.5 mm)	17-28.5 mm (\bar{X} 23.75 mm)	83-137 mm (\bar{X} 100 mm)	28-29 mm (\bar{X} 28.5 mm)	-
Number of	29-41	28-35	34-38	31-32	-

<i>annuli</i>	(\bar{X} 31.6)	(\bar{X} 31)	(\bar{X} 36.5)	(\bar{X} 31.5)	
Diameter of cephalothorax	4-5.5 mm (\bar{X} 4.75 mm)	2-2.5 mm (\bar{X} 2 mm)	4 mm	-	-
Neck	2-3 mm (\bar{X} 2.5 mm)	-	2 mm	-	-
Diameter of abdomen	3.5-5 mm (\bar{X} 4.2 mm)	1.8-2.2 mm (\bar{X} 2 mm)	4 mm	-	-
Hook parameter:			-	-	-
a. AC					
b. CB	0.23-0.55 mm	0.22-0.35 mm			
c. AD	0.37-0.69 mm 0.47-0.88 mm	0.29-0.35 mm 0.29-0.45 mm			
Mouth:			-	-	-
a. Length	0.16-29 mm	0.14-0.38 mm			
b. Width	0.12-0.24 mm	0.12-0.18 mm			
Egg:		-	-	-	
a. Length	114-130 μ m (\bar{X} 118 μ m)				125-154 μ m (\bar{X} 133 μ m)
b. Width	108-124 μ m (\bar{X} 116 μ m)				106-143 μ m (\bar{X} 123 μ m)

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138 **Figure 2.** Differences in size between female (top) and male (bellow).
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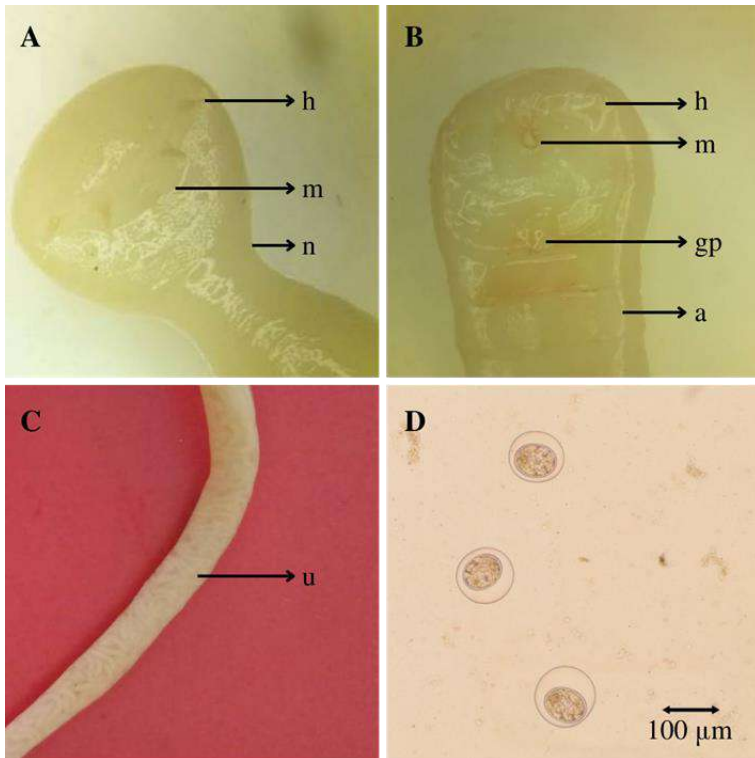
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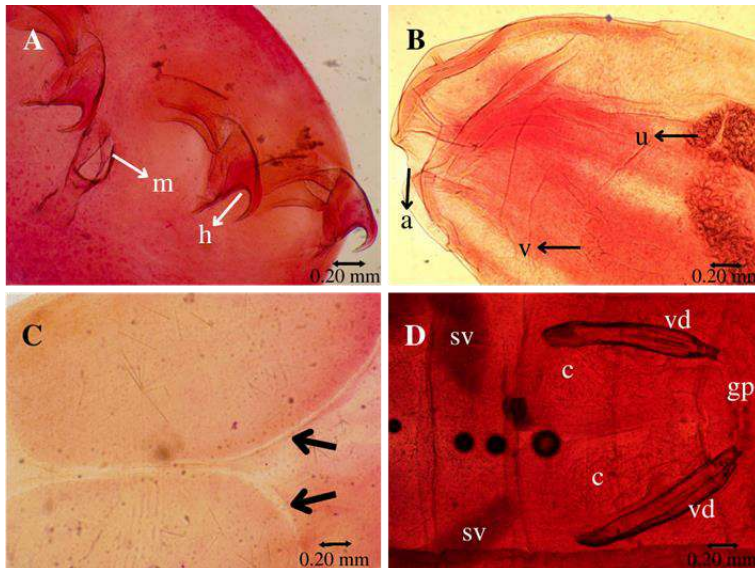
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Both sexes, adult males and female pentastomids, were found in this research (Figures 1 and 2). The number of male pentastomid was less (21 heads) than that of females (183 heads) Table 2. Table 2 also describes the size of each pentastomid. The female pentastomids had longer and larger bodies than the males (Figures 1 and 2). The length of the female bodies was 72-125 mm with an average of 94.5 mm. Their abdominal diameter was 3.5-5 mm with an average of 4.2 mm. Furthermore, the anterior section or cephalothorax of the females was globular with a neck separating their head and their abdomen (Figure 3A). The average length and width of the female mouths were 0.24 mm and 0.16 mm. Furthermore, the results of the microscopy examination using Carmine staining showed that the female pentastomid had two spermathecae on the anterior part that connect directly to the uterus that contains the egg (Figures 3C, 4B, and 4C). Vagina was in the posterior body and around the anus (Figure 4B).



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151 **Figure 3.** Pentastomida without staining. (A) The anterior section of the female. (B) The anterior section of the male. (C) Female body.
152 (D) Pentastomid's eggs. h: hook, m: mouth, n: neck, gp: genital pore, a: first annuli, u: uterus.
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154 On the other hand, the length of the males' bodies was 17-28.5 mm with an average of 23.75 mm. Their abdominal
155 diameter was 1.8-2.2 mm with an average of 2 mm. The average length and width of the mouth were 0.23 and 0.14 mm,
156 respectively. The male body was simple and straight without any curves in the neck (Figure 3B). The genital pore was
157 identified in the first annulus and there was a pair of seminal vesicles, a pair of vasa deferens and cirrus (4D).
158



159
160 **Figure 4.** Carmine-stained pentastomida. (A) The anterior section. (B) The posterior section of the female. (C) A pair of female
161 spermatheca. (D) Male reproductive organs. m: mouth, h: hook, a: anus, v: vagina, u: uterus, sv: seminal vesicle, c: cirrus, vd: vas
162 deferens.
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164 The eggs were oval and consisted of two layers of membrane that clearly could be distinguished. The first layer (outer
165 membrane) was thin, while the second layer (inner membrane) was thick and yellowish. There was fluid-filled space
166 between the first and second layers. The length of the eggs was 114-130 µm with an average of 118 µm and the width was
167 108-124 µm with an average of 116 µm (Figure 3D).

168 Pentastomida were mostly found in the lungs and the parasites also spread to the trachea in five snakes. This finding
169 was similar to research by Riley and Self (1980) that revealed that adult *Kiricephalus pattoni* (one of the pentastomida

170 species) were only found in the lungs of *P. mucosa* snakes. In our observation, pentastomids that spread to the trachea
171 were females. According to Sundar et al. (2015), the adult pentastomids with mature eggs are expelled from the trachea
172 and eliminated from the definitive host by oral expulsion or may also be swallowed, resulting in shed eggs in the feces in
173 which another intermediate mammal host will ingest to continue their life cycles.

174 The number of male pentastomids was fewer than the number of female ones. This case was also experienced by John
175 and Nadakal (1988) and Riley and Self (1980) that only found a few males compared the female. We agree with Almeida
176 et al. (2006) that pentastomids are not well known, in biology and the number of their species.

177 The taxonomy of pentastomida is still controversial. It has been known through molecular data that pentastomida are
178 closely related to crustaceans and belong to class Maxillopoda, sub-phylum Crustacea, and the phylum Arthropoda (Paré,
179 2008). Based on the size of the egg (Almeida et al., 2007), the form of the female head that is globular with a neck
180 separating the head from its body (Paré, 2008) and the size of the body length, the number of the annuli, the diameter of
181 cephalothorax, neck, abdominal diameter, head, and body shape (Riley and Self, 1979; Riley and Self, 1980), the
182 pentastomida found was classified as *K. pattoni*. *K. pattonii* can be classified into the order of porocephalida (Paré, 2008).
183 According to Christoffersen and De Assis (2013; 2015), pentastomid in *P. mucosa* snakes are classified into *K. pattoni*.

184 *K. pattoni* has a long annulated body, a tapered tail, an anal canal, two pairs of hooks or a total of four hooks on its
185 head, as well as a small ovoid-shaped mouth (Riley and Self, 1979). The female *K. pattoni* has a larger body than male one
186 (Paré, 2008). Female *K. pattoni* also has a spiral body with a larger head than its body, and a curve of the neck, whereas
187 male one has a straight body without a curve on the neck (Riley and Self, 1979; Riley and Self, 1980; John and Nadakal,
188 1988; Almeida et al., 2007). The spiral body in females is likely to facilitate their movement into the lung tissue of the
189 definitive hosts (John and Nadakal, 1988).

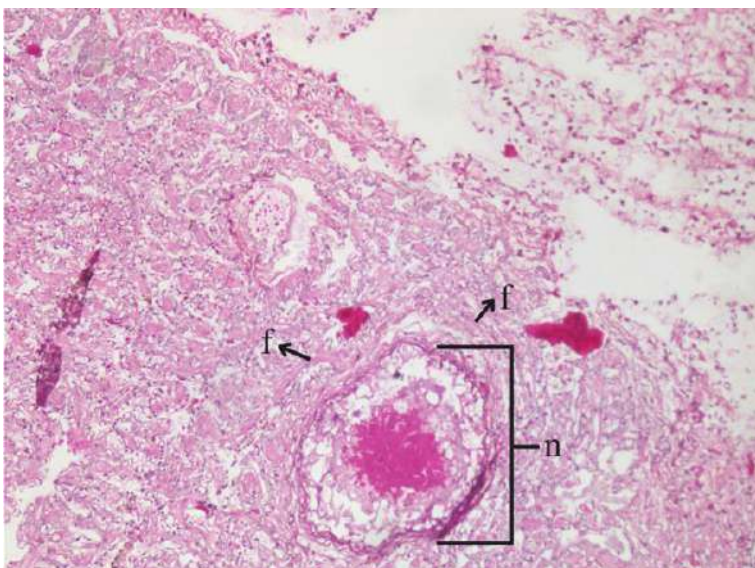
190 The morphology of the eggs like the eggs of *Porocephalus crotali* described by Sundar et al. (2015), the eggs were
191 composed of two-layer membranes of which the outer one is thin, and the inner membrane thick and there was a fluid-
192 filled space between two membranes. But the average diameter of the eggs measured in this investigation is smaller (118 x
193 116 µm) than the eggs of *P. crotali* (144 µm) as described by Sundar et al. (2015).

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195 **Histopathological changes in the lungs of *Ptyas mucosa***

196 Squamates typically have two lungs, however, the left lung in most snakes is greatly reduced, never exceeding 85% of
197 the right lung's size, or absent [JB27]. The right lung usually runs from near the heart to the cranial to the [right kidn [JB28]ey.
198 The cranial portion of a lung is vascularized and functions primarily as a gas exchanger (vascular lung), whereas the
199 caudal primarily functions as an air sac (saccular lung). The vascular lung's wall is made up of honeycombed units called
200 faveoli, which allow for gas exchange (Funk and Bogan, 2019). Microscopically, the faveoli in the snake lung parenchyma
201 are lined mainly by squamous epithelial cells (type I cells) and, to a lesser degree, by cuboidal epithelial cells (type II)
202 (Jacobson et al., 2021).

203 In this study, a nymphal stage of the parasite was found in the pulmonary tissue infected with pentastomida. The
204 parasite is surrounded by some connective tissue (Figure 5). Finding the stages of the nymph in the histological lung of the
205 snake and the stages of the parasite on the macroscopic lung organ means that the *P. mucosa* snake may act as an
206 intermediate and final host of *K. pattoni*. Within the lung cavities of the definitive host, the nymphs develop into adults
207 (Sundar et al., 2015). In this study, pentastomida infection in the lungs only caused a mild histological reaction in the form
208 of fibrosis in the area around the parasite.
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Figure 5. Histological microscopic picture of nymph-infected pulmo from pentastomida showed the presence of fibroblast around the area of infection. f: fibroblast, n: the body of the nymph stage pentastomida.

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Pentastomida have an indirect life cycle that typically utilizes vertebrate hosts. Many of their species rely on reptiles as their definitive host, and the adults are usually found in the lungs (Wellehan and Walden, 2019). Pentastomida parasites migrate extensively through the visceral before settling in the lungs and, less typically, the trachea and nasal passageways. Pentastomida can produce localized lesions, whilst molting can elicit antigenic reactions. Adults feed on lung tissue fluids and pass embryonated eggs, which are coughed up through the oral cavity, swallowed, and passed in the feces (Comolli and Divers, 2021). In squamates, the pulmonary lumen is large, and the tracheal diameter may be smaller than that of the pentastomids. This poses a risk of respiratory obstruction by the adult pentastomes. However, the greatest pathological changes are seen in the intermediate host, where they may become embedded in tissues (Wellehan and Walden, 2019[JB29]).

Pentastomiasis in reptiles, especially snakes, occurs subclinically, although the infection is large, and death is usually associated with severe pneumonia[JB30]. Because pentastomids attach to the lung tissue, they can cause perforation, hemorrhaging, and serious inflammation in the host (Fernando and Fernando, 2014). In a Common Garter Snake (*Thamnophis sirtalis*), lung lesions were associated with one of the pentastomida species, *Kiricephalus coarctatus*, where the embedded parasite had gotten into the hypaxial muscle. Infections with *Armillifer armillatus* have also been linked to parasitic pneumonia in *Gaboon vipers*. Pentastomids occluding the trachea have been implicated in snake deaths. Bacterial infections could develop due to lung damage. Four of nine *Boa constrictors* with pulmonary damage from *Porocephalus dominicana* infection perished from bacteremia and pericarditis (Walden et al., 2021).

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CONCLUSION

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The prevalence of pentastomiasis in slaughtered *Ptyas mucosa* in Sidoarjo was 65% (39 of 60 snakes). The pentastomids that infect these snakes are classified as *Kiricephalus pattoni*. The pulmonary histological findings only show a light reaction in the form of mild fibrosis.

235

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236

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1 **First Report of Pentastomiasis and the Lung Histopathological Changes** 2 **in Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia**

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12 **Abstract.** Currently, reptiles, especially snakes, are increasingly popular as pets around the world and are often consumed by several
13 communities in Indonesia. This trend needs attention from a public health perspective because it has the potential to cause zoonotic diseases,
14 one of which is parasitic disease, pentastomiasis. This study aimed to determine the prevalence of pentastomiasis, identify the parasite,
15 and observe the pulmonary histopathology in Oriental rat snakes (*Ptyas mucosa*) collected in Tulangan, Sidoarjo, Indonesia. The
16 methods used were the calculation of the prevalence of pentastomiasis, semichen-acetic carmine staining, and identification of parasites
17 and eggs based on references as a comparison. Furthermore, histopathological preparation and hematoxylin-eosin staining were
18 performed on the lungs infected with the parasite to observe the microscopic lesions. The results showed that 65% of the snakes had
19 pentastomiasis, and the parasites were characterized as *Kirichephalus pattoni*. Microscopical examination revealed mild fibrosis in the
20 pulmonary area as a result of pentastomid infestation. This is the first reported study of pentastomiasis in *P. mucosa* in Sidoarjo,
21 Indonesia, with the implication that the snakes that were caught and commonly consumed were infected with zoonotic pentastomides.
22 **Key words:** Colubrid Snake, Parasitic Pneumonia, Tongue Worms, Zoonotic Parasitoses.

23 **Abbreviations:**

24 **Running title:** Pentastomiasis in Rat Snake

25 INTRODUCTION

26 Exotic animals such as reptiles, especially various types of snakes, are now popular as pets throughout the world
27 (Okulewicz et al. 2014). However, they are also concerned with the increasing animal welfare and health issues through
28 the animal trade in the market (Yudhana et al. 2021). Some species of snake are bred in captivity, while others are caught
29 in the wild, and sold as pets or as bushmeat for consumption (Hardi et al. 2017; Yudhana et al. 2021).

30 *Ptyas mucosa* is a well-known snake species found in Indonesia (Pranashinta et al. 2017). This type of snake, known as
31 the Oriental rat snake and Indian rat snake, belongs to the Colubridae family which is widely distributed throughout East,
32 South, and Southeast Asia (Auliya 2010; Muliya and Bhat 2016; Yuan et al. 2022). *P. mucosa* is a diurnal and semi-
33 arboreal snake species that lives on forest floors, agricultural fields, wet ground, and in close proximity to human habitats.
34 It feeds on frogs, rats, and other small animals (Chittora et al. 2021).

35 The meat of *P. mucosa* is used in the illegal trade to China (Auliya 2010). The meat and internal organs are used as
36 fresh or frozen food, the bile and blood are used in traditional medicine, and the skin is exported to several European
37 countries as raw materials for the leather industry (Pranashinta et al. 2017). Although it is illegal, most rural communities
38 in Indonesia trade and consume the meat and bile of *P. mucosa* for some health reasons (Auliya 2010; Pranashinta et al.
39 2017).

40 Wildlife meat, however, poses a threat of transmitting infection with novel and exotic pathogens to human hosts (Hardi
41 et al. 2017). Then new zoonotic diseases are likely to emerge consequently. For instance, Bats (Chiroptera) are important
42 reservoirs of several emerging diseases, including Ebola virus disease (EVD), severe acute respiratory syndrome (SARS),
43 and Middle East respiratory syndrome (MERS), as well as the current pandemic COVID-19 caused by SARS Coronavirus
44 2 (Irving et al. 2021). Many types of parasites are also the cause of zoonotic emerging diseases, resulting in a significant
45 public health burden, particularly in tropical countries (Alasil and Abdullah 2019).

46 Several parasites found in confined reptiles spend a portion of their life cycle in the respiratory system. The majority of
47 respiratory involvement is asymptomatic and mild, generating mostly localized inflammation, with the exception of
48 instances with excessive parasite loads. However, severe disease can result from high burdens or unusual host-parasite
49 interactions, which is frequently made worse by secondary infections (Comolli 2021). Reptile-borne pentastomiasis is an
50 unusual tropical disease that is neglected and under-researched in Asia, Africa, and South America (Sulyok et al. 2014).

51 Pentastomiasis is a parasitic zoonosis caused by infection with pentastomids, belonging to the subclass Pentastomida,
52 under the phylum Arthropoda (Chittora et al. 2021; Tongtako et al. 2013; WHA 2019). Pentastomida are also known as
53 tongue worms since the adult phase of the genus *Linguatula* has similarities with the mammalian tongues (Paré 2008).

54 Adult parasites are usually found in the respiratory tract of reptiles, birds, and mammals, including humans, while their
55 larval forms are in the internal organs of insects, mammals, and amphibians (Paré 2008; Chen et al. 2010; Kelehear et al.
56 2014). Some zoonotic pentastomida in snakes are *Armillifer* and *Porocephalus crotali* (Rataj et al. 2011). Humans can be
57 infected with pentastomids since they consume raw snake meat, have direct contact with the snake, drink water
58 contaminated with parasitic eggs, or are contaminated through the feces (Kelehear et al. 2014; Tappe et al. 2016;
59 Mendoza-Roldan et al. 2020).

60 Pentastomiasis in snakes have been reported in many countries, such as poisonous *Terciopelo* (*Bothrops asper*) in
61 Costa Rica (Alvarado et al. 2015), *Royal python* (*Python regius*) snakes in Nigeria (Ayinmode et al. 2010), *Rat snakes*
62 (*Ptyas mucosa*) in Sri Lanka and India (Fernando and Fernando 2014; Sundar et al. 2015), wild snakes in Australia
63 (Kelehear et al. 2014), various species of snakes in Slovenia and Pakistan (Rataj et al. 2011), *Chinese moccasin*
64 (*Agkistrodon acutus*) and *Indian python* (*Python molurus*) in China (Chen et al. 2010), and *Totonacan rattlesnake*
65 (*Crotalus totonacus*) in Mexico (Tejos-Ramírez et al. 2022).

66 To the best of our knowledge, there are no reports of pentastomids infections in snakes in Indonesia. Therefore, this
67 study reports the prevalence of pentastomiasis, the identification of pentastomids, and the pulmonary histopathological
68 profiles in *Oriental rat snakes* (*P. mucosa*) in Sidoarjo Sub-district, East Java, Indonesia.

69 MATERIALS AND METHODS

70 Ethical approval

71 This study was reviewed and approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine,
72 Universitas Airlangga (number 678-KE).

73 Parasite collection

74 The sample used comes from the *Oriental rat snake* (*Ptyas mucosa*), which is often caught and sold for its meat and
75 skin through collectors. The collection was conducted at a snake slaughterhouse in Tulangan District, Sidoarjo Regency,
76 East Java Province, Indonesia. The presence of pentastomid parasites was observed in the respiratory tract of sixty
77 slaughtered snakes. Pentastomides were taken and cleaned with distilled water, then stored in sterile plastic pots. The lungs
78 of the snakes infected with the parasite were also isolated for histopathological preparation.

80 Prevalence calculation

81 The prevalence of pentastomiasis in snake samples was calculated by the following formula:

82
$$\text{Prevalence (\%)} = (\text{The number of snakes infected with pentastomids}) / (\text{Total sample of snakes}) \times 100\%.$$

84 Semichen-acetic carmine staining

85 The collected pentastomids were stained with the Semichen-Acetic Carmine technique (Kuhlmann 2006). Adult
86 pentastomids were fixed on object glasses and soaked in 5% glycerin alcohol for 24 hours. Then they were soaked in 70%
87 alcohol for five minutes. Subsequently, the object glasses with pentastomids were placed in Carmine alcohol solution (1:2)
88 for eight hours. The pentastomids were soaked in acid alcohol for 10 minutes and moved to base alcohol for 20 minutes.
89 Furthermore, graded dehydration was carried out with 70%, 85% and 95% alcohol for five minutes, respectively. The
90 mounting step was done using Hung's I for 20 minutes. Pentastomids were taken and placed on new clean object glasses.
91 The pentastomids were dripped with Hung's II solution and then covered with a cover glass.

93 Parasite identification

94 Characteristics of the head and body shape and body size of the parasite were identified based on Riley and Self
95 (1980), Almedia et al. (2006), and Christoffersen and De Assis (2013; 2015). Identification of parasite egg size was carried
96 out according to Keegan (1943).

97 The length of the fresh parasites was measured from the anterior edge to the posterior end. The head was observed
98 under a stereo microscope and documented with a Canon Digital Single-Lens Reflex (DSLR) camera. Stained parasites
99 were observed using a light microscope with 40-100× magnification and documented using Miconos Optilab. Some
100 female parasites were incubated in PBS overnight at 37 °C to collect the eggs. The supernatants were centrifuged at 1,500
101 rpm for 10 minutes and the pellet was dripped onto a glass object and observed using a light microscope with 100×
102 magnification. Eggs were measured using Miconos Optilab.

104 Histopathological preparations

105 The lungs of snakes with pentastomid infestation were fixed in 10% formalin. Then graded dehydration was carried out
106 with serial alcohol concentrations (70%, 80%, 90%, 96%, and absolute) and clearing using xylol. Furthermore, paraffin
107 embedding was performed to obtain a solid block of tissue. The tissue blocks were sliced (3 micrometers thick) using a
108 microtome, mounted on an object glass, and stained using Hematoxylin and Eosin. Microscopic examination was
109 performed using Nikon Eclipse Ci.

111

RESULTS AND DISCUSSIONS

The prevalence of pentastomiasis in *Ptyas mucosa* snakes

112 Thirty-nine or 65% of the respiratory tract of 60 were positively infected with adult pentastomids. In total, 204
 113 pentastomids were collected (Table 1). Pentastomida were found to be strongly attached to the trachea and lungs of snakes
 114 (Figure 1). Some pentastomids even buried their heads in the lungs of snakes. The parasites of all snakes positively
 115 infected with pentastomids were found in the lungs (39 snakes), while only 5 snakes which parasites spread to their
 116 tracheas.
 117
 118

119 **Table 1.** Prevalence of pentastomiasis in *Ptyas mucosa* based on site and number of parasites.

Sites	Positive (%)	Number of parasites
Lungs only	57% (34 of 60)	2-9 with the average of 5 pentastomida
Trachea only	0% (0 of 60)	-
Lungs and Trachea	8% (5 of 60)	7-9 with the average of 8 pentastomida
Total	65% (39 of 60)	204 pentastomida

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121

122 **Figure 1.** Pentastomid infestation in the lungs of *Ptyas mucosa*. The white arrows show areas with raised lesions around the attachment
 123 of pentastomids to the tissue. The black arrows show female pentastomids and the red arrow shows male pentastomids.
 124

125 This is the first study to determine the prevalence of pentastomiasis in *Ptyas mucosa* in Sidoarjo, East Java Indonesia.
 126 As shown in our results, pentastomids were present in 39 of 60 (65%) snakes. Based on this finding, the prevalence of
 127 pentastomiasis in *P. mucosa* in this study was quite high. In this case, it was similar as the prevalence of pentastomiasis in
 128 Brazil, 8 of 15 or 53.33% of snakes were positively infected with pentastomid. The high prevalence in this research
 129 probably caused by the food consumed by the snakes. The type of food is very influential on the prevalence of infection
 130 and the type of pentastomida that infected the snakes (Almeida et al. 2007). *P. mucosa* snakes usually feed on rats, frogs,
 131 toads, lizards, and even smaller non-venomous and smaller snakes (Sidik 2006). According to Esslinger (1962), rats, frogs,
 132 toads, lizards, and even non-venomous snakes can act as the intermediate hosts of pentastomida.
 133

Identification of pentastomida

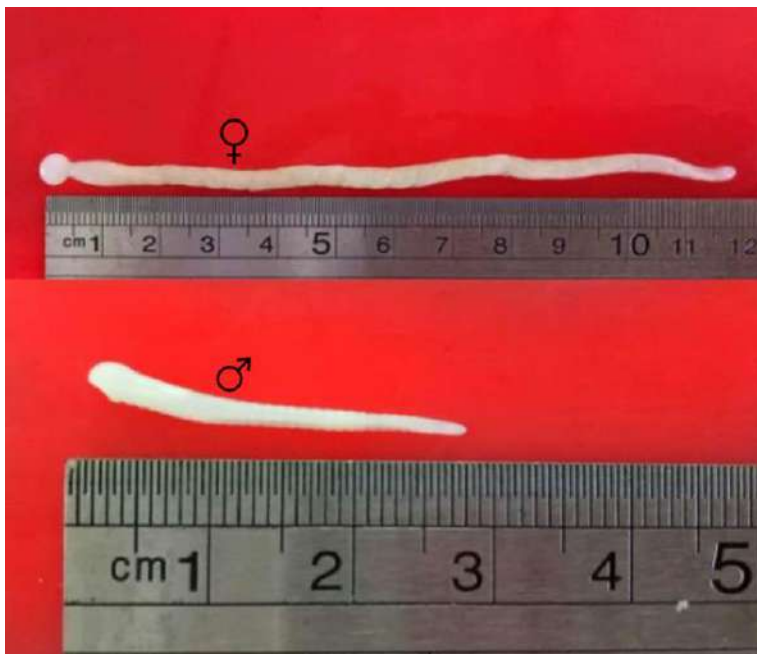
134 The characteristics of pentastomid found in this study can be seen in Table 2 and Figure 2. Pentastomids have a long
 135 cylindrical body with a larger head, a flat shape on the ventral, and a tapered tail or posterior. Their body was soft and
 136 whitish with a colorless cuticle, which tends to be transparent so that the internal organs are visible. There were annuli
 137 throughout their body up to the posterior. There are two pairs of hooks (a total of four hooks) around the head that are used
 138 to attach to the host's lung tissue, and it had an ovoid-shaped mouth between the two pairs of hooks.
 139

141 **Table 2.** The characteristics of pentastomida compared with *Kiricephalus pattoni* by Riley and Self (1980) and Keegan
 142 (1943).

Parameters	The current study		British Museum collection (Riley and Self 1980)		Keegan 1943
	Females	Males	Females	Males	
Total number of parasites	183	21	17	2	-
Body length	72-125 mm (\bar{X} 94.5 mm)	17-28.5 mm (\bar{X} 23.75 mm)	83-137 mm (\bar{X} 100 mm)	28-29 mm (\bar{X} 28.5 mm)	-
Number of annuli	29-41 (\bar{X} 31.6)	28-35 (\bar{X} 31)	34-38 (\bar{X} 36.5)	31-32 (\bar{X} 31.5)	-

Diameter of cephalothorax	4-5.5 mm (\bar{X} 4.75 mm)	2-2.5 mm (\bar{X} 2 mm)	4 mm	-	-
Neck	2-3 mm (\bar{X} 2.5 mm)	-	2 mm	-	-
Diameter of abdomen	3.5-5 mm (\bar{X} 4.2 mm)	1.8-2.2 mm (\bar{X} 2 mm)	4 mm	-	-
Hook parameter:			-	-	-
a. AC					
b. CB	0.23-0.55 mm	0.22-0.35 mm			
c. AD	0.37-0.69 mm 0.47-0.88 mm	0.29-0.35 mm 0.29-0.45 mm			
Mouth:			-	-	-
a. Length	0.16-29 mm	0.14-0.38 mm			
b. Width	0.12-0.24 mm	0.12-0.18 mm			
Egg:		-	-	-	-
a. Length	114-130 μ m (\bar{X} 118 μ m)				125-154 μ m (\bar{X} 133 μ m)
b. Width	108-124 μ m (\bar{X} 116 μ m)				106-143 μ m (\bar{X} 123 μ m)

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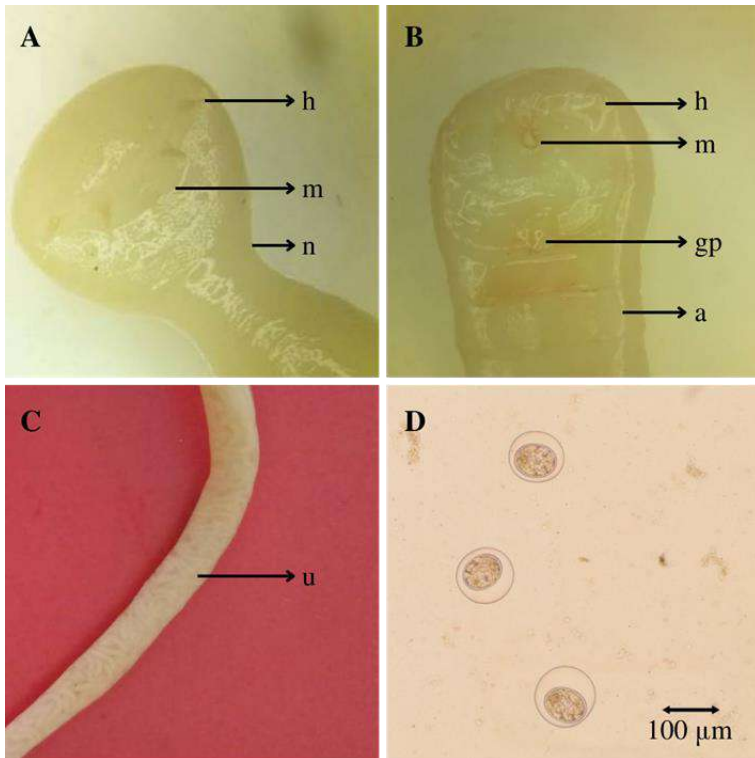
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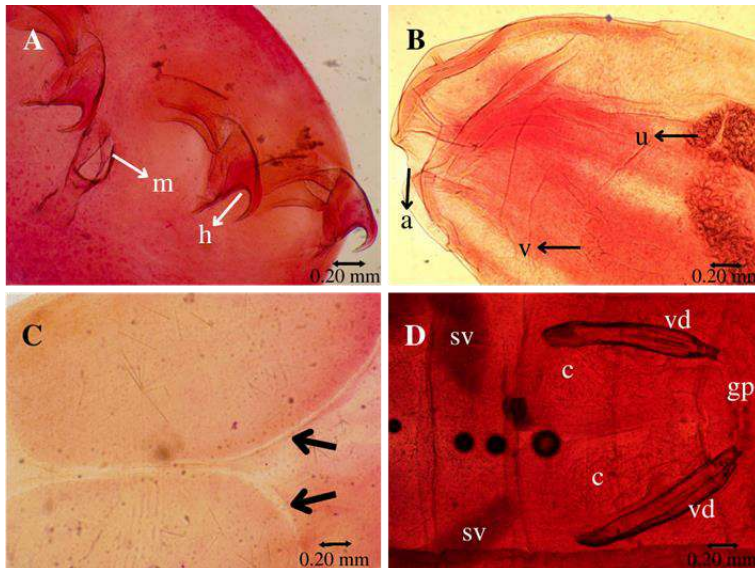
Figure 2. Differences in size between female (top) and male (bellow).

Both sexes, adult males and female pentastomids, were found in this research (Figures 1 and 2). The number of male pentastomid was less (21 heads) than that of females (183 heads) Table 2. Table 2 also describes the size of each pentastomid. The female pentastomids had longer and larger bodies than the males (Figures 1 and 2). The length of the female bodies was 72-125 mm with an average of 94.5 mm. Their abdominal diameter was 3.5-5 mm with an average of 4.2 mm. Furthermore, the anterior section or cephalothorax of the females was globular with a neck separating their head and their abdomen (Figure 3A). The average length and width of the female mouths were 0.24 mm and 0.16 mm. Furthermore, the results of the microscopy examination using Carmine staining showed that the female pentastomid had two spermathecae on the anterior part that connect directly to the uterus that contains the egg (Figures 3C, 4B, and 4C). Vagina was in the posterior body and around the anus (Figure 4B).



157
158 **Figure 3.** Pentastomida without staining. (A) The anterior section of the female. (B) The anterior section of the male. (C) Female body.
159 (D) Pentastomid's eggs. h: hook, m: mouth, n: neck, gp: genital pore, a: first annuli, u: uterus.
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161 On the other hand, the length of the males' bodies was 17-28.5 mm with an average of 23.75 mm. Their abdominal
162 diameter was 1.8-2.2 mm with an average of 2 mm. The average length and width of the mouth were 0.23 and 0.14 mm,
163 respectively. The male body was simple and straight without any curves in the neck (Figure 3B). The genital pore was
164 identified in the first annulus and there was a pair of seminal vesicles, a pair of vasa deferens and cirrus (4D).
165



166 **Figure 4.** Carmine-stained pentastomida. (A) The anterior section. (B) The posterior section of the female. (C) A pair of female
167 spermatheca. (D) Male reproductive organs. m: mouth, h: hook, a: anus, v: vagina, u: uterus, sv: seminal vesicle, c: cirrus, vd: vas
168 deferens.
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171 The eggs were oval and consisted of two layers of membrane that clearly could be distinguished. The first layer (outer
172 membrane) was thin, while the second layer (inner membrane) was thick and yellowish. There was fluid-filled space
173 between the first and second layers. The length of the eggs was 114-130 µm with an average of 118 µm and the width was
174 108-124 µm with an average of 116 µm (Figure 3D).

175 Pentastomida were mostly found in the lungs and the parasites also spread to the trachea in five snakes. This finding
176 was similar to research by Riley and Self (1980) that revealed that adult *Kiricephalus pattoni* (one of the pentastomida

177 species) were only found in the lungs of *P. mucosa* snakes. In our observation, pentastomids that spread to the trachea
178 were females. According to Sundar et al. (2015), the adult pentastomids with mature eggs are expelled from the trachea
179 and eliminated from the definitive host by oral expulsion or may also be swallowed, resulting in shed eggs in the feces in
180 which another intermediate mammal host will ingest to continue their life cycles.

181 The number of male pentastomids was fewer than the number of female ones. This case was also experienced by John
182 and Nadakal (1988) and Riley and Self (1980) that only found a few males compared the female. We agree with Almeida
183 et al. (2006) that pentastomids are not well known, in biology and the number of their species.

184 The taxonomy of pentastomida is still controversial. It has been known through molecular data that pentastomida are
185 closely related to crustaceans and belong to class Maxillopoda, sub-phylum Crustacea, and the phylum Arthropoda (Paré
186 2008). Based on the size of the egg (Almeida et al. 2007), the form of the female head that is globular with a neck
187 separating the head from its body (Paré 2008) and the size of the body length, the number of the annuli, the diameter of
188 cephalothorax, neck, abdominal diameter, head, and body shape (Riley and Self 1979; Riley and Self 1980), the
189 pentastomida found was classified as *K. pattoni*. *K. pattonii* can be classified into the order of porochepalida (Paré 2008).
190 According to Christoffersen and De Assis (2013; 2015), pentastomid in *P. mucosa* snakes are classified into *K. pattoni*.

191 *K. pattoni* has a long annulated body, a tapered tail, an anal canal, two pairs of hooks or a total of four hooks on its
192 head, as well as a small ovoid-shaped mouth (Riley and Self, 1979). The female *K. pattoni* has a larger body than male one
193 (Paré 2008). Female *K. pattoni* also has a spiral body with a larger head than its body, and a curve of the neck, whereas
194 male one has a straight body without a curve on the neck (Riley and Self 1979; Riley and Self 1980; John and Nadakal
195 1988; Almeida et al. 2007). The spiral body in females is likely to facilitate their movement into the lung tissue of the
196 definitive hosts (John and Nadakal 1988).

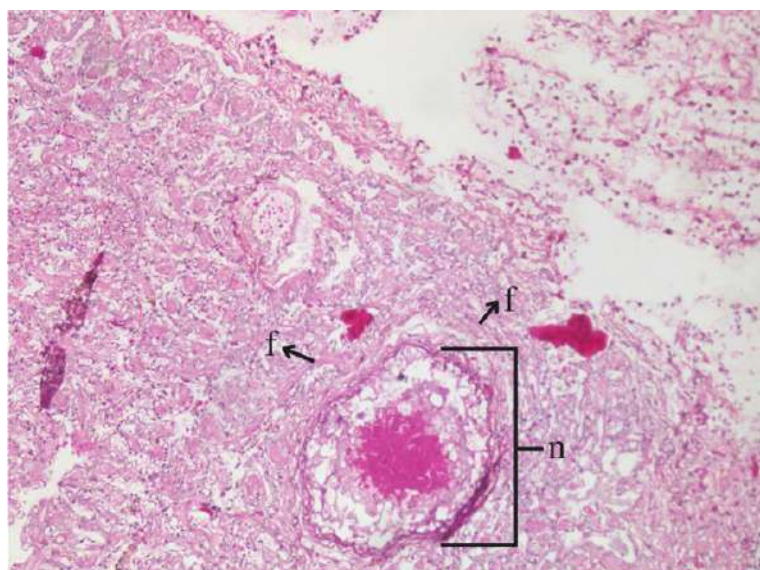
197 The morphology of the eggs like the eggs of *Porocephalus crotali* described by Sundar et al. (2015), the eggs were
198 composed of two-layer membranes of which the outer one is thin, and the inner membrane thick and there was a fluid-
199 filled space between two membranes. But the average diameter of the eggs measured in this investigation is smaller (118 x
200 116 µm) than the eggs of *P. crotali* (144 µm) as described by Sundar et al. (2015).

202 **Histopathological changes in the lungs of *Ptyas mucosa***

203 Squamates typically have two lungs, however, the left lung in most snakes is greatly reduced, never exceeding 85% of
204 the right lung's size, or absent (Funk and Bogan 2019). The right lung usually runs from near the heart to the cranial to the
205 right kidney (Funk and Bogan 2019). The cranial portion of a lung is vascularized and functions primarily as a gas
206 exchanger (vascular lung), whereas the caudal primarily functions as an air sac (saccular lung). The vascular lung's wall is
207 made up of honeycombed units called faveoli, which allow for gas exchange (Funk and Bogan 2019). Microscopically, the
208 faveoli in the snake lung parenchyma are lined mainly by squamous epithelial cells (type I cells) and, to a lesser degree, by
209 cuboidal epithelial cells (type II) (Jacobson et al. 2021).

210 In this study, a nymphal stage of the parasite was found in the pulmonary tissue infected with pentastomida. The
211 parasite is surrounded by some connective tissue (Figure 5). Finding the stages of the nymph in the histological lung of the
212 snake and the stages of the parasite on the macroscopic lung organ means that the *P. mucosa* snake may act as an
213 intermediate and final host of *K. pattoni*. Within the lung cavities of the definitive host, the nymphs develop into adults
214 (Sundar et al. 2015). In this study, pentastomida infection in the lungs only caused a mild histological reaction in the form
215 of fibrosis in the area around the parasite.

216



217 **Figure 5.** Histological microscopic picture of nymph-infected pulmo from pentastomida showed the presence of fibroblast around the
218 area of infection. f: fibroblast, n: the body of the nymph stage pentastomida.
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Pentastomida have an indirect life cycle that typically utilizes vertebrate hosts. Many of their species rely on reptiles as their definitive host, and the adults are usually found in the lungs (Wellehan and Walden 2019). Pentastomida parasites migrate extensively through the visceral before settling in the lungs and, less typically, the trachea and nasal passageways. Pentastomida can produce localized lesions, whilst molting can elicit antigenic reactions. Adults feed on lung tissue fluids and pass embryonated eggs, which are coughed up through the oral cavity, swallowed, and passed in the feces (Comolli and Divers 2021). In squamates, the pulmonary lumen is large, and the tracheal diameter may be smaller than that of the pentastomids. This poses a risk of respiratory obstruction by the adult pentastomes. However, the greatest pathological changes are seen in the intermediate host, where they may become embedded in tissues (Wellehan and Walden 2019).

Pentastomiasis in reptiles, especially snakes, occurs subclinically, although the infection is large, and death is usually associated with secondary septicemia and pneumonia (Tappe and Büttner 2009). Because pentastomids attach to the lung tissue, they can cause perforation, hemorrhaging, and serious inflammation in the host (Fernando and Fernando 2014). In a Common Garter Snake (*Thamnophis sirtalis*), lung lesions were associated with one of the pentastomida species, *Kiricephalus coarctatus*, where the embedded parasite had gotten into the hypaxial muscle. Infections with *Armillifer armillatus* have also been linked to parasitic pneumonia in *Gaboon vipers*. Pentastomids occluding the trachea have been implicated in snake deaths. Bacterial infections could develop due to lung damage. Four of nine *Boa constrictors* with pulmonary damage from *Porocephalus dominicana* infection perished from bacteremia and pericarditis (Walden et al. 2021).

Bigger implications, in terms of possible impacts on human health, may be anticipated based on the histopathological changes found in this study. Humans are the final hosts of pentastomes, which can be contracted from improperly cooked meat from infected snakes or from drinking water contaminated with pentastomida eggs. In addition, humans have the possibility of getting the infection when handling and harvesting snake skin or having direct contact with the mouth of a pet snake (Latif et al. 2016). The pathological consequences to the clinical symptoms experienced by infected humans may be the same or even more severe, depending on the number of parasite eggs ingested, the duration from the larvae migrating to various organs to maturity and reproduction, and how severe the parasite infestation is in the human viscera.

Personal hygiene measures, such as avoiding drinking river water directly or boiling the water before drinking, are required to prevent human infections. Authorities should provide health education to warn people about eating undercooked snake meat, the possibility of transmission after handling snakes, and avoiding contact with snake excretions.

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CONCLUSION

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The prevalence of pentastomiasis in slaughtered *Ptyas mucosa* in Sidoarjo was 65% (39 of 60 snakes). The pentastomids that infect these snakes are classified as *Kiricephalus pattoni*. The pulmonary histological findings only show a light reaction in the form of mild fibrosis.

252

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1 First report of pentastomiasis and the lung histopathological changes in 2 Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia 3 4 5 6 7 8 9 10 11

12 **Abstract.** Currently, reptiles, especially snakes, are increasingly popular as pets ~~around the world~~ worldwide and are often consumed by
13 several communities in Indonesia. This trend needs attention from a public health perspective because it ~~has the potential to can~~ potentially
14 cause zoonotic diseases, ~~one of which is including~~ parasitic disease, and pentastomiasis. This study aimed to determine: the prevalence of
15 pentastomiasis, identify the parasite, and observe the pulmonary histopathology in Oriental rat snakes (*Ptyas mucosa*) collected in
16 Tulangan, Sidoarjo, Indonesia. The methods used were ~~the calculation of the prevalence of pentastomiasis, semichen-acetic carmine~~
17 ~~staining, and identification of calculating the prevalence of pentastomiasis, semichen-acetic carmine staining, and identifying~~ parasites
18 and eggs based on references as a comparison. Furthermore, histopathological preparation and hematoxylin-eosin staining were
19 performed on the lungs infected with the parasite to observe the microscopic lesions. The results showed that 65% of the snakes had
20 pentastomiasis, and the parasites were ~~characterized as called~~ *Kirichephalus pattoni*. ~~Microscopical~~ Furthermore, a ~~microscopical~~
21 examination revealed mild fibrosis in the pulmonary area ~~due to~~ as a result of pentastomid infestation. ~~This study~~ is the first reported
22 ~~study of~~ pentastomiasis in *P. mucosa* in Sidoarjo, Indonesia, ~~showing with the implication that~~ that the snakes ~~that were~~ caught and
23 commonly consumed were infected with zoonotic pentastomid ~~omices~~.
24 **Key-words:** Colubrid Snake, Parasitic Pneumonia, Tongue Worms, Zoonotic Parasitoses.

25 **Abbreviations:**

26 **Running title:** Pentastomiasis in Rat Snake

27 INTRODUCTION

28 Exotic animals such as reptiles, especially various ~~types of~~ snakes, are now popular as pets ~~throughout the~~
29 ~~world~~ worldwide (Okulewicz et al. 2014). However, they are also concerned with the increasing animal welfare and health
30 issues through the animal trade in the market (Yudhana et al. 2021). Some species of snake are bred in captivity, while
31 others are caught in the wild, and sold as pets or ~~as~~ bushmeat for consumption (Hardi et al. 2017; Yudhana et al. 2021).

32 *Ptyas mucosa* is a well-known snake species ~~found~~ in Indonesia (Pranashinta et al. 2017). This type of snake, known as
33 the Oriental rat snake and Indian rat snake, belongs to the Colubridae family which is widely distributed throughout East,
34 South, and Southeast Asia (Auliya 2010; Muliya and Bhat 2016; Yuan et al. 2022). *P. mucosa* is a diurnal and semi-
35 arboreal snake species that lives on forest floors, agricultural fields, wet ground, and ~~in close proximity to near~~
36 human habitats. It feeds on frogs, rats, and other small animals (Chittora et al. 2021).

37 The meat of *P. mucosa* is used in the illegal trade to China (Auliya 2010). The meat and internal organs are used as
38 fresh or frozen food, the bile and blood are used in traditional medicine, and the skin is exported to several European
39 countries as raw materials for the leather industry (Pranashinta et al. 2017). Although ~~it is~~ illegal, most rural communities
40 in Indonesia trade and consume the meat and bile of *P. mucosa* for some ~~health~~ reasons (Auliya 2010; Pranashinta et al.
41 2017).

42 Wildlife meat, however, poses a threat of transmitting infection with novel and exotic pathogens to human hosts (Hardi
43 et al. 2017). Then new zoonotic diseases are likely to emerge consequently. For instance, Bats (Chiroptera) are important
44 reservoirs of several emerging diseases, including Ebola virus disease (EVD), ~~S~~evere acute respiratory syndrome (SARS),
45 and Middle East respiratory syndrome (MERS), as well as the current pandemic COVID-19 caused by SARS Coronavirus
46 2 (Irving et al. 2021). ~~Moreover, many types of parasites are also the cause of~~ ~~also cause zoonotic emerging~~
47 ~~zoonotic~~ diseases, resulting in a significant public health burden, particularly in tropical countries (Alasil and Abdullah,
48 2019).

49 Several parasites ~~found~~ in confined reptiles spend a portion of their life cycle in the respiratory system. ~~The majority of~~
50 ~~respiratory involvement is asymptomatic and mild, generating mostly localized inflammation, with the exception of~~ Most
51 ~~respiratory involvement is asymptomatic and mild, generating mostly localized inflammation except for instances with~~
52 excessive parasite loads. However, severe disease can result from high burdens or unusual host-parasite interactions, ~~which~~

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53 is frequently ~~made worse~~worsened by secondary infections (Comolli 2021). Reptile-borne pentastomiasis is an unusual
54 tropical disease ~~that is~~ neglected and under-researched in Asia, Africa, and South America (Sulyok et al. 2014).

55 Pentastomiasis is a parasitic zoonosis caused by infection with ~~pentastomids~~pentatomic, belonging to the subclass
56 Pentastomida, under the phylum Arthropoda (Chittora et al. 2021; Tongtako et al. 2013; WHA 2019). Pentastomi~~asida~~ ~~are~~
57 ~~is~~ also known as tongue worms since the adult phase of the genus *Linguatula* has similarities with ~~the~~ mammalian tongues
58 (Paré 2008). Adult parasites are usually found in the respiratory tract of reptiles, birds, and mammals, including humans,
59 while their larval forms are in the internal organs of insects, mammals, and amphibians (Paré 2008; Chen et al. 2010;
60 Kelehear et al. 2014). Some zoonotic ~~pentastomida~~Pentastomida in snakes ~~are is~~ *Armillifer* and *Porocephalus crotali*
61 (Rataj et al. 2011). Humans can be infected with ~~pentastomids~~pentatomic ~~after~~sinee they consume raw snake meat, have
62 direct contact with the snake, drink water contaminated with parasitic eggs, or are contaminated through the feces
63 (Kelehear et al. 2014; Tappe et al. 2016; Mendoza-Roldan et al. 2020).

64 Pentastomiasis in snakes have been reported in many countries, such as poisonous Terciopelo (*Bothrops asper*) in
65 Costa Rica (Alvarado et al. 2015), Royal python (*Python regius*) snakes in Nigeria (Ayinmode et al. 2010), Rat snakes
66 (*Ptyas mucosa*) in Sri Lanka and India (Fernando and Fernando 2014; Sundar et al. 2015), wild snakes in Australia
67 (Kelehear et al. 2014), various species of snakes in ~~Slovenia~~Slovenia and Pakistan (Rataj et al. 2011), Chinese moccasin
68 (*Agkistrodon acutus*) and Indian python (*Python molurus*) in China (Chen et al. 2010), and Totonacan rattlesnake
69 (*Crotalus tonotacus*) in Mexico (Tepos-Ramírez et al. 2022).

70 ~~Moreover,~~ ~~to the best of our best~~ knowledge, ~~there are no reports of pentastomids infections in snakes in~~
71 ~~Indonesia~~snakes in Indonesia have no reports of pentatomic infections. ~~Therefore,~~ ~~therefore,~~ this study reporteds the
72 prevalence ~~and of~~ pentastomiasis, ~~the~~ ~~identification~~ ~~pentastomidaes of~~ pentastomids, and the pulmonary
73 histopathological profiles in Oriental rat snakes (*P. mucosa*) in Sidoarjo Sub-district, East Java, Indonesia.

74 MATERIALS AND METHODS

75 Ethical approval

76 ~~This study was reviewed and approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine,~~
77 ~~Universitas Airlangga (number 678-KE) Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas~~
78 ~~Airlangga (number 678-KE) reviewed and approved this study.~~

80 Parasite collection

81 The sample used comes from the Oriental rat snake (*Ptyas mucosa*), which is often caught and sold for its meat and
82 skin through collectors. The collection was conducted at a snake slaughterhouse in Tulangan District, Sidoarjo Regency,
83 East Java Province, Indonesia. The presence of pentastomid parasites was observed in the respiratory tract of ~~60~~sixty
84 slaughtered snakes. Pentastomides were taken and cleaned with distilled water, then stored in sterile plastic pots. The lungs
85 of the snakes infected with the parasite were also isolated for histopathological preparation.

87 Prevalence calculation

88 ~~The prevalence of pentastomiasis in snake samples was calculated by the following formula~~following formula
89 ~~calculated the prevalence of pentastomiasis in snake samples:~~

$$90 \text{Prevalence (\%)} = (\text{The number of snakes infected with pentastomids}) / (\text{Total sample of snakes}) \times 100\%.$$

92 Semichen-acetic carmine staining

93 The collected pentastomids were stained with the Semichen-Acetic Carmine technique (Kuhlmann 2006). Adult
94 pentastomids were fixed on object glasses and soaked in 5% glycerin alcohol for 24 hours. Then they were soaked in 70%
95 alcohol for five minutes. Subsequently, the object glasses with pentastomids were placed in Carmine alcohol solution (1:2)
96 for eight hours. ~~The Next,~~ the pentastomids were soaked in acid alcohol for 10 minutes and moved to base alcohol for 20
97 minutes. Furthermore, graded dehydration was carried out with 70%, 85%, and 95% alcohol for five minutes, ~~respectively.~~
98 The mounting step was done using ~~Hung's~~Hung's I for 20 minutes. ~~Pentastomids~~Finally, pentastomids were taken and
99 placed on new clean object glasses. The pentastomids were dripped with ~~Hung's~~Hung's II solutions and then covered with
100 a cover glass.

102 Parasite identification

103 Characteristics of the head and body shape and body size of the parasite were identified based on Riley and Self
104 (1980), Almedia et al. (2006), and Christoffersen and De Assis (2013; 2015). Identification of parasite egg size was carried
105 out according to Keegan (1943).

106 The ~~length of the~~fresh parasite's length was measured from the anterior edge to the posterior end. The head was
107 observed under a stereo microscope and documented with a Canon Digital Single-Lens Reflex (DSLR) camera.
108 ~~Furthermore,~~ stained parasites were observed using a light microscope with 40-100× magnification and documented
109 using Miconos Optilab. Some female parasites were incubated in PBS overnight at 37 °C to collect the eggs. The

supernatants were centrifuged at 1,500 rpm for 10 minutes, and the pellet was dripped onto a glass object and observed using a light microscope with 100× magnification. Those eggs were then measured using Miconos Optilab.

Histopathological preparations

The lungs of snakes with pentastomid infestation were fixed in 10% formalin. Then graded dehydration was carried out with serial alcohol concentrations (70%, 80%, 90%, 96%, and absolute) and clearing using xylol. Furthermore, paraffin embedding was performed to obtain a solid block of tissue. The tissue blocks were sliced (3 micrometers thick) using a microtome, mounted on an object glass, and stained using Hematoxylin and Eosin. Microscopic examination was performed using Nikon Eclipse Ci.

RESULTS AND DISCUSSIONS

The prevalence of pentastomiasis in *Ptyas mucosa* snakes

Thirty-nine (or 65%) of the respiratory tract of 60 samples were positively infected with adult pentastomids; a total of 204 pentastomids were collected (Table 1). Pentastomida were found to be strongly attached to the trachea and lungs of snakes (Figure 1). Some pentastomids even buried their heads in the lungs of snakes. The parasites of all snakes positively infected with pentastomids were found in the lungs (39 snakes), while only five snakes which parasites spread to their tracheas.

Table 1. Prevalence of pentastomiasis in *Ptyas mucosa* based on site and the number of parasites.

Sites	Positive (%)	Number of parasites
Lungs only	57% (34 of 60)	2-9 with the average of 5 pentastomida
Trachea only	0% (0 of 60)	-
Lungs and Trachea	8% (5 of 60)	7-9 with the average of 8 pentastomida
Total	65% (39 of 60)	204 pentastomida



Figure 1. Pentastomid infestation in the lungs of *Ptyas mucosa*. The white arrows show areas with raised lesions around the attachment of pentastomids to the tissue. The black arrows show female pentastomids, and the red arrow shows male pentastomids.

This is the first study to determine the prevalence of pentastomiasis in *Ptyas mucosa* in Sidoarjo, East Java, Indonesia. As shown in our results, our results showed that pentastomids were present in 39 of 60 (65%) snakes (Table 1). Based on this finding, the prevalence of pentastomiasis in *P. mucosa* in this study was quite high. In this study, it was similar as the prevalence is similar to pentastomiasis in Brazil, that 8 of 15 or 53.33% of snakes were positively infected with pentastomida. The high prevalence in this research was probably caused by the food consumed by the snakes; food consumed by the snakes probably caused the high prevalence in this research. The type of food is very influential on the prevalence of infection and the type of pentastomida that infected the snakes (Almeida et al. 2007). *P. mucosa* snakes usually feed on rats, frogs, toads, lizards, and even smaller non-venomous and smaller snakes (Sidik 2006). In addition, according to Esslinger (1962), rats, frogs, toads, lizards, and even non-venomous snakes can act as the intermediate hosts of pentastomida.

Identification of pentastomida

The characteristics of pentastomid found in this study can be seen in Table 2 and Figure 2. Pentastomids have a long cylindrical body with a larger head, a flat shape on the ventral, and a tapered tail or posterior. Their body was soft and whitish with a colorless cuticle, which tends to be transparent so that the internal organs are visible. There were annuli throughout their body up to the posterior. There are two pairs of hooks (a total of four

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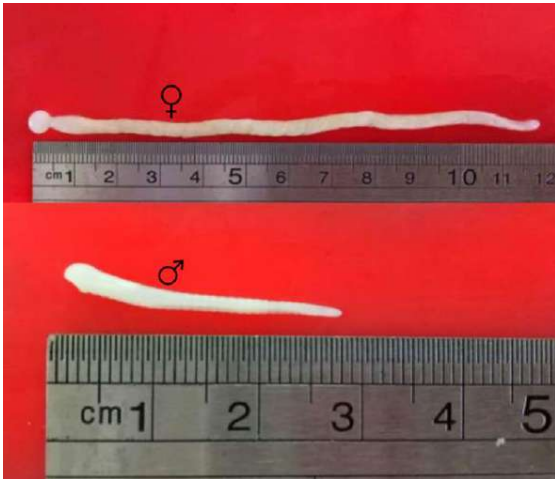
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149 hooks) around the head that are used to attach to the host's host's lung tissue, and it had-has an ovoid-shaped mouth
 150 between the two pairs of hooks.
 151

152 **Table 2.** The characteristics of pentastomida compared with *Kiricephalus pattoni* by Riley and Self (1980) and Keegan
 153 (1943).

Parameters	The current study		British Museum collection (Riley and Self 1980)		Keegan 1943
	Females	Males	Females	Males	
Total number of parasites	183	21	17	2	-
Body length	72-125 mm (\bar{X} 94.5 mm)	17-28.5 mm (\bar{X} 23.75 mm)	83-137 mm (\bar{X} 100 mm)	28-29 mm (\bar{X} 28.5 mm)	-
Number of annuli	29-41 (\bar{X} 31.6)	28-35 (\bar{X} 31)	34-38 (\bar{X} 36.5)	31-32 (\bar{X} 31.5)	-
Diameter of cephalothorax	4-5.5 mm (\bar{X} 4.75 mm)	2-2.5 mm (\bar{X} 2 mm)	4 mm	-	-
Neck	2-3 mm (\bar{X} 2.5 mm)	-	2 mm	-	-
Diameter of abdomen	3.5-5 mm (\bar{X} 4.2 mm)	1.8-2.2 mm (\bar{X} 2 mm)	4 mm	-	-
Hook parameter:					
a. AC			-	-	-
b. CB	0.23-0.55 mm	0.22-0.35 mm			
c. AD	0.37-0.69 mm	0.29-0.35 mm			
	0.47-0.88 mm	0.29-0.45 mm			
Mouth:					
a. Length	0.16-29 mm	0.14-0.38 mm	-	-	-
b. Width	0.12-0.24 mm	0.12-0.18 mm			
Egg:					
a. Length	114-130 μ m (\bar{X} 118 μ m)				125-154 μ m (\bar{X} 133 μ m)
b. Width	108-124 μ m (\bar{X} 116 μ m)				106-143 μ m (\bar{X} 123 μ m)

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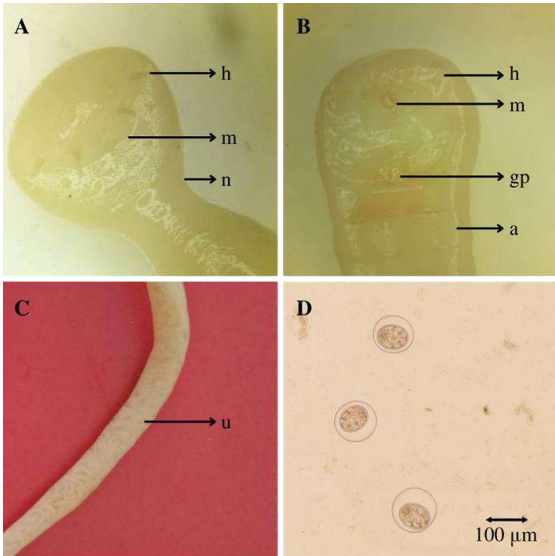


155 **Figure 2.** Differences in size between females (top) and males (below).
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158 Both sexes, adult males and female pentastomids, were found in this research (Figures 1 and 2). The number of male
 159 pentastomid was less (21 heads) than that of females (183 heads) Table 2. Table 2 also describes the size of each
 160 pentastomid. The female pentastomids had longer and larger bodies than the males (Figures 1 and 2). The length of the

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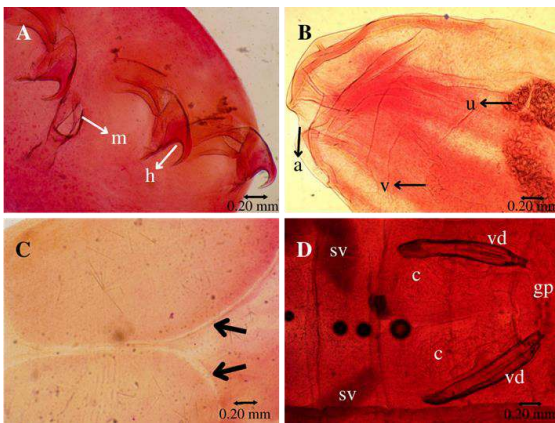
female bodies was 72-125 mm, with an average of 94.5 mm. Their abdominal diameter was 3.5-5 mm ~~with an average of,~~ ~~averaging~~ 4.2 mm. Furthermore, the anterior section or cephalothorax of the females was globular, with a neck separating their head and ~~their~~ abdomen (Figure 3A). The average length and width of the female mouths were 0.24 mm and 0.16 mm. Furthermore, the results of the microscopy examination using Carmine staining showed that the female pentastomid had two spermathecae on the anterior part. ~~That connects~~ directly to the uterus that contains the egg (Figures 3C, 4B, and 4C). ~~In addition, the v~~agina was in the posterior body and around the anus (Figure 4B).



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Figure 3. Pentastomida without staining. (A) The anterior section of the female. (B) The anterior section of the male. (C) Female body. (D) Pentastomid's Pentastomid's eggs. h: hook, m: mouth, n: neck, gp: genital pore, a: first annuli, u: uterus.

On the other hand, the length of the ~~males'-males'~~ bodies was 17-28.5 mm, with an average of 23.75 mm. Their abdominal diameter was 1.8-2.2 mm ~~with an average of,~~ ~~averaging~~ 2 mm. The average length and width of the mouth were 0.23 and 0.14 mm, respectively. The male body was simple and straight without any curves in the neck (Figure 3B). The genital pore was identified in the first annulus, and there was a pair of seminal vesicles, ~~a pair of vasa~~ deferens, and cirrus (4D).



178

179 **Figure 4.** Carmine-stained pentastomida. (A) The anterior section. (B) The posterior section of the female. (C) A pair of female
180 spermatheca. (D) Male reproductive organs. m: mouth, h: hook, a: anus, v: vagina, u: uterus, sv: seminal vesicle, c: cirrus, vd: vas
181 deferens.

182
183 The eggs were oval and consisted of two layers of membrane that clearly could be distinguished. The first layer (outer
184 membrane) was thin, while the second layer (inner membrane) was thick and yellowish. There was fluid-filled space
185 between the first and second layers. The length of the eggs was 114-130 μm with an average of 118 μm , and the width was
186 108-124 μm with an average of 116 μm (Figure 3D).

187 Pentastomida were/was mostly found in the lungs, and the parasites also spread to the trachea in five snakes. This
188 finding was similar to research by Riley and Self (1980) that revealed that adult *Kiricephalus pattoni* (one of the
189 pentastomida species) were only found in the lungs of *P. mucosa* snakes. In our observation, pentastomids that spread to
190 the trachea were females. According to Sundar et al. (2015), the adult pentastomids with mature eggs are expelled from the
191 trachea and eliminated from the definitive host by oral expulsion or may also be swallowed. That results, resulting in shed
192 eggs in the feces, in which another intermediate mammal host will ingest to continue their life cycles.

193 The number of male pentastomids was fewer than the number of female ones. This case was also experienced by John
194 and Nadakal (1988) and Riley and Self (1980), that who only found a few males compared the female. This study result
195 follows We agree with Almeida et al. (2006), whose number of pentastomids and that pentastomids are not well known, in
196 biology and the number of their species their species is unknown in biology.

197 The taxonomy of pentastomida is still controversial. Moreover, i-It has been known through molecular data that
198 pentastomida are closely related to crustaceans and belong to the class Maxillopoda, sub-phylum Crustacea, and the
199 phylum Arthropoda (Paré 2008). Based on the size of the egg size (Almeida et al. 2007); the form of the female head form
200 that is globular with a neck separating the head from its body (Paré 2008); and the size of the body length, the number of
201 the annuli, the diameter of the cephalothorax, neck, abdominal diameter, head, and body shape (Riley and Self 1979; Riley
202 and Self 1980); the pentastomida found was classified as *K. pattoni*. *K. pattoni* can be classified into the order of as
203 Pporocephalida (Paré 2008). In addition, aAccording to Christoffersen and De Assis (2013; 2015), pentastomid in *P.*
204 *mucosa* snakes are classified into *K. pattoni*.

205 *K. pattoni* has a long annulated body, a tapered tail, an anal canal, two pairs of hooks or a total of four hooks on its
206 head, as well as four hooks on its head, and a small ovoid-shaped mouth (Riley and Self, 1979). The female *K. pattoni* has
207 a larger body than the male one (Paré 2008). Female *K. pattoni* also has a spiral body with a larger head than its body, and
208 a curve of the neck, whereas male one has a straight body without a curve on the neck (Riley and Self 1979; Riley and Self
209 1980; John and Nadakal 1988; Almeida et al. 2007). The spiral body in females is likely to facilitate their movement into
210 the lung tissue of the definitive hosts (John and Nadakal 1988).

211 The egg morphology is similar of the eggs liketo the eggs of *Porocephalus crotali* described by Sundar et al. (2015). T,
212 thesee eggs were composed of two-layer membranes, of which the outer one is-was thin, and the inner membrane thick,
213 and there was a fluid-filled space between those two membranes. But the average diameter of the eggs measured in this
214 investigation is smaller (118 x 116 μm) than the eggs of *P. crotali* (144 μm), as described by Sundar et al. (2015).

215 **Histopathological changes in the lungs of *Ptyas mucosa***

216 Squamates typically have two lungs-; however, the left lung in most snakes is greatly reduced, never exceeding 85% of
217 the right lung's-lung's size, or absent-absence (Funk and Bogan 2019). The right lung usually runs from near the heart to
218 the cranial to the right kidney (Funk and Bogan 2019). The cranial portion of a lung is vascularized and functions
219 primarily as a gas exchanger (vascular lung), whereas the caudal primarily functions as an air sac (saccular lung). The
220 vascular lung's wall is-made-up-ofcomprises honeycombed units called faveoli, which allow-forallowing gas exchange
221 (Funk and Bogan 2019).-Microscopically, Tthe faveoli in the snake lung parenchyma are lined mainly-microscopically
222 by squamous epithelial cells (type I cells). In addition and, to a lesser degree, by cuboidal epithelial cells (type II) (Jacobson
223 et al. 2021).

224 In this study, a nymphal stage of the parasite was found in the pulmonary tissue infected with pentastomida. The
225 parasite is surrounded by some connective tissue (Figure 5). Finding the stages of the nymph in the histological lung of the
226 snake and the stages of the parasitesnake's histological lung and the parasite's stages on-in the macroscopic lung organ
227 means that the *P. mucosa* snake may act as an intermediate and final host of *K. pattoni*. Within the lung cavities of the
228 definitive host, the nymphs develop into adults (Sundar et al. 2015). In this study, pentastomida infection in the lungs only
229 caused a mild histological reaction in the form of fibrosis in the area around the parasite.

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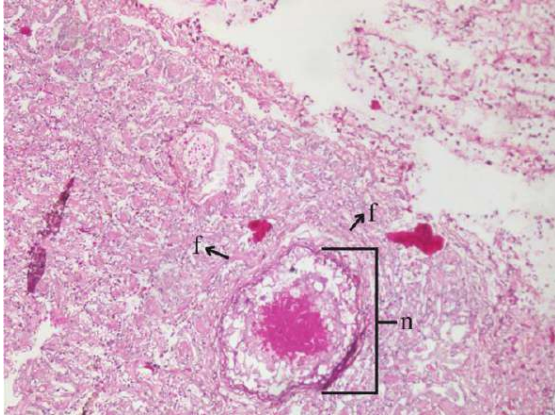


Figure 5. Histological microscopic picture of nymph-infected pulmo-Pulmo from pentastomida showed the presence of fibroblast around the area of infection. f: fibroblast, n: the body of the nymph stage pentastomida.

Pentastomida have an indirect life cycle that typically utilizes vertebrate hosts. Consequently, these species rely on reptiles as their definitive host, and the adults are usually found in their lungs (Wellehan and Walden 2019). Pentastomida parasites migrate extensively through the visceral before settling in the lungs and, less typically, the trachea and nasal passageways. Pentastomida can produce localized lesions, whilst molting can elicit antigenic reactions. Adults feed on lung tissue fluids and pass embryonated eggs, which are coughed up through the oral cavity, swallowed, and passed in the feces (Comolli and Divers 2021). In squamates, the pulmonary lumen is large, and the tracheal diameter may be smaller than that of the pentastomids. This poses a risk of respiratory obstruction by the adult pentastomides. However, the greatest pathological changes are seen in the intermediate host, where they may become embedded in tissues (Wellehan and Walden 2019).

Pentastomiasis in reptiles, especially snakes, occurs subclinically. Although the infection is large, and death is usually associated with secondary septicemia and pneumonia (Tappe and Büttner 2009). Because pentastomids attach to the lung tissue, they can cause perforation, hemorrhaging, and serious inflammation in the host (Fernando and Fernando 2014). In a Common Garter Snake (*Thamnophis sirtalis*), lung lesions were associated with one of the pentastomida species, *Kiricephalus coarctatus*, where the embedded parasite had gotten into the hypaxial muscle. Infections with *Armillifer armillatus* have also been linked to parasitic pneumonia in *Gaboon vipers*. Pentastomids occluding the trachea have been implicated in snake deaths. Bacterial infections could develop due to lung damage. For example, there are four out of nine *Boa constrictors* with pulmonary damage from *Porocephalus dominicana* infection, perished due to bacteremia and pericarditis (Walden et al. 2021).

Bigger implications, in terms of regarding possible impacts on human health, may be anticipated based on the histopathological changes found in this study. Humans are the final hosts of pentastomes, which can be contracted from improperly cooked meat from infected snakes or from drinking water contaminated with pentastomida eggs. In addition, humans have the possibility of getting the infection when handling and harvesting snake-skin or having direct contact with the mouth of a pet snake (Latif et al. 2016). The pathological consequences of the clinical symptoms experienced by infected humans may be the same or even more severe. That, depending on the number of parasite eggs ingested, the duration from the larvae migrating to various organs to maturity and reproduction, and how severe the parasite infestation is in the human viscera.

Personal hygiene measures, such as avoiding drinking river water directly or boiling the water before drinking, are required to prevent human infections. For example, avoid drinking river water directly or boiling the water before drinking. Authorities, in addition, should provide health education to warn people about eating undercooked snake meat, the possibility of transmission after handling snakes, and avoiding contact with snake excretions.

CONCLUSION

The prevalence of pentastomiasis in slaughtered *Ptyas mucosa* in Sidoarjo was 65% (39 of 60 snakes). The pentastomids that infect these snakes are classified as *Kiricephalus pattoni*. The pulmonary histological findings only show a light reaction in the form of mild fibrosis.

270

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271

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272

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1 **First report of pentastomiasis and the lung histopathological changes in** 2 **Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia** 3 4 5 6 7 8 9 10 11

12 **Abstract.** Currently, reptiles, especially snakes, are increasingly popular as pets worldwide and are often consumed by several communities
13 in Indonesia. This trend needs attention from a public health perspective because it can potentially cause zoonotic diseases, including
14 parasitic disease and pentastomiasis. This study aimed to determine: the prevalence of pentastomiasis, identify the parasite, and observe
15 the pulmonary histopathology in Oriental rat snakes (*Ptyas mucosa*) collected in Tulangan, Sidoarjo, Indonesia. The methods used were
16 calculating the prevalence of pentastomiasis, semichon-acetic carmine staining, and identifying parasites and eggs based on references
17 as a comparison. Furthermore, histopathological preparation and hematoxylin-eosin staining were performed on the lungs infected with
18 the parasite to observe the microscopic lesions. The results showed that 65% of the snakes had pentastomiasis, and the parasites were
19 called *Kirichephalus pattoni*. Furthermore, a microscopical examination revealed mild fibrosis in the pulmonary area due to pentastomid
20 infestation. This study is the first reported pentastomida in *P. mucosa* in Sidoarjo, Indonesia, showing that snakes caught and commonly
21 consumed were infected with zoonotic pentastomic.

22 **Key-words:** Colubrid Snake, Parasitic Pneumonia, Tongue Worms, Zoonotic Parasitoses.

23 **Abbreviations:**

24 **Running title:** Pentastomiasis in Rat Snake

25 **INTRODUCTION**

26 Exotic animals such as reptiles, especially various snakes, are now popular as pets worldwide (Okulewicz et al. 2014).
27 However, they are also concerned with the increasing animal welfare and health issues through the animal trade in the
28 market (Yudhana et al. 2021). Some species of snake are bred in captivity, while others are caught in the wild and sold as
29 pets or bushmeat for consumption (Hardi et al. 2017; Yudhana et al. 2021).

30 *Ptyas mucosa* is a well-known snake species in Indonesia (Pranashinta et al. 2017). This type of snake, known as the
31 Oriental rat snake and Indian rat snake, belongs to the Colubridae family which is widely distributed throughout East,
32 South, and Southeast Asia (Auliya 2010; Muliya and Bhat 2016; Yuan et al. 2022). *P. mucosa* is a diurnal and semi-
33 arboreal snake species that live on forest floors, agricultural fields, wet ground, and near human habitats. It feeds on frogs,
34 rats, and other small animals (Chittora et al. 2021).

35 The meat of *P. mucosa* is used in the illegal trade to China (Auliya 2010). The meat and internal organs are used as
36 fresh or frozen food, the bile and blood are used in traditional medicine, and the skin is exported to several European
37 countries as raw materials for the leather industry (Pranashinta et al. 2017). Although illegal, most rural communities in
38 Indonesia trade and consume the meat and bile of *P. mucosa* for some reasons (Auliya 2010; Pranashinta et al. 2017).

39 Wildlife meat, however, poses a threat of transmitting infection with novel and exotic pathogens to human hosts (Hardi
40 et al. 2017). Then new zoonotic diseases are likely to emerge consequently. For instance, Bats (Chiroptera) are important
41 reservoirs of several emerging diseases, including Ebola virus disease (EVD), Severe acute respiratory syndrome (SARS),
42 and Middle East respiratory syndrome (MERS), as well as the current pandemic COVID-19 caused by SARS Coronavirus
43 2 (Irving et al. 2021). Moreover, many parasites also cause emerging zoonotic diseases, resulting in a significant public
44 health burden, particularly in tropical countries (Alasil and Abdullah 2019).

45 Several parasites in confined reptiles spend a portion of their life cycle in the respiratory system. Most respiratory
46 involvement is asymptomatic and mild, generating mostly localized inflammation except for excessive parasite loads.
47 However, severe disease can result from high burdens or unusual host-parasite interactions, frequently worsened by
48 secondary infections (Comolli 2021). Reptile-borne pentastomiasis is an unusual tropical disease neglected and under-
49 researched in Asia, Africa, and South America (Sulyok et al. 2014).

50 Pentastomiasis is a parasitic zoonosis caused by infection with pentastomic, belonging to the subclass Pentastomida,
51 under the phylum Arthropoda (Chittora et al. 2021; Tongtako et al. 2013; WHA 2019). Pentastomiasis is also known as
52 tongue worms since the adult phase of the genus *Linguatula* has similarities with mammalian tongues (Paré 2008). Adult
53 parasites are usually found in the respiratory tract of reptiles, birds, and mammals, including humans, while their larval

54 forms are in the internal organs of insects, mammals, and amphibians (Paré 2008; Chen et al. 2010; Kelehear et al. 2014).
55 Some zoonotic Pentastomida in snakes is Armillifer and *Porocephalus crotali* (Rataj et al. 2011). Humans can be infected
56 with pentatomic after they consume raw snake meat, have direct contact with the snake, drink water contaminated with
57 parasitic eggs, or are contaminated through the feces (Kelehear et al. 2014; Tappe et al. 2016; Mendoza-Roldan et al.
58 2020).

59 Pentastomiasis in snakes have been reported in many countries, such as poisonous Terciopelo (*Bothrops asper*) in
60 Costa Rica (Alvarado et al. 2015), Royal python (*Python regius*) snakes in Nigeria (Ayinmode et al. 2010), Rat snakes
61 (*Ptyas mucosa*) in Sri Lanka and India (Fernando and Fernando 2014; Sundar et al. 2015), wild snakes in Australia
62 (Kelehear et al. 2014), various species of snakes in Slovenia and Pakistan (Rataj et al. 2011), Chinese moccasin
63 (*Agkistrodon acutus*) and Indian python (*Python molurus*) in China (Chen et al. 2010), and Totonacan rattlesnake
64 (*Crotalus totonacus*) in Mexico (Tepos-Ramírez et al. 2022).

65 Moreover, to our best knowledge, snakes in Indonesia have no reports of pentatomic infections. Therefore, this study
66 reported the prevalence and identified pentastomida and the pulmonary histopathological profiles in Oriental rat snakes
67 (*P. mucosa*) in Sidoarjo Sub-district, East Java, Indonesia.

68

MATERIALS AND METHODS

69 Ethical approval

70 The Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga (number 678-KE)
71 reviewed and approved this study.

72

73 Parasite collection

74 The sample used comes from the Oriental rat snake (*Ptyas mucosa*), which is often caught and sold for its meat and
75 skin through collectors. The collection was conducted at a snake slaughterhouse in Tulangan District, Sidoarjo Regency,
76 East Java Province, Indonesia. The presence of pentastomid parasites was observed in the respiratory tract of 60
77 slaughtered snakes. Pentastomides were taken and cleaned with distilled water, then stored in sterile plastic pots. The lungs
78 of the snakes infected with the parasite were also isolated for histopathological preparation.

79

80 Prevalence calculation

81 The following formula calculated the prevalence of pentastomiasis in snake samples:

82
$$\text{Prevalence (\%)} = (\text{The number of snakes infected with pentastomids}) / (\text{Total sample of snakes}) \times 100\%.$$

83

84 Semichen-acetic carmine staining

85 The collected pentastomids were stained with the Semichen-Acetic Carmine technique (Kuhlmann 2006). Adult
86 pentastomids were fixed on object glasses and soaked in 5% glycerin alcohol for 24 hours. Then they were soaked in 70%
87 alcohol for five minutes. Subsequently, the object glasses with pentastomids were placed in Carmine alcohol solution (1:2)
88 for eight hours. Next, the pentastomids were soaked in acid alcohol for 10 minutes and moved to base alcohol for 20
89 minutes. Furthermore, graded dehydration was carried out with 70%, 85%, and 95% alcohol for five minutes. The
90 mounting step was done using Hung's I for 20 minutes. Finally, pentastomids were taken and placed on new clean object
91 glasses. The pentastomids were dripped with Hung's II solutions and then covered with a cover glass.

92

93 Parasite identification

94 Characteristics of the head and body shape and body size of the parasite were identified based on Riley and Self
95 (1980), Almedia et al. (2006), and Christoffersen and De Assis (2013; 2015). Identification of parasite egg size was carried
96 out according to Keegan (1943).

97 The fresh parasite's length was measured from the anterior edge to the posterior end. The head was observed under a
98 stereo microscope and documented with a Canon Digital Single-Lens Reflex (DSLR) camera. Furthermore, stained
99 parasites were observed using a light microscope with 40-100× magnification and documented using Miconos Optilab.
100 Some female parasites were incubated in PBS overnight at 37 °C to collect the eggs. The supernatants were centrifuged at
101 1,500 rpm for 10 minutes, and the pellet was dripped onto a glass object and observed using a light microscope with 100×
102 magnification. Those eggs were then measured using Miconos Optilab.

103

104 Histopathological preparations

105 The lungs of snakes with pentastomid infestation were fixed in 10% formalin. Then graded dehydration was carried out
106 with serial alcohol concentrations (70%, 80%, 90%, 96%, and absolute) and clearing using xylol. Furthermore, paraffin
107 embedding was performed to obtain a solid block of tissue. The tissue blocks were sliced (3 micrometers thick) using a
108 microtome, mounted on an object glass, and stained using Hematoxylin and Eosin. Microscopic examination was
109 performed using Nikon Eclipse Ci.

110

RESULTS AND DISCUSSIONS

The prevalence of pentastomiasis in *Ptyas mucosa* snakes

112 Thirty-nine (or 65%) of the respiratory tract of 60 samples were positively infected with adult pentastomids; a total of
 113 204 pentastomids samples were collected (Table 1). Pentastomida was found to be strongly attached to the trachea and
 114 lungs of snakes (Figure 1). Some pentastomids even buried their heads in the lungs of snakes. The parasites of all snakes
 115 positively infected with pentastomids were found in the lungs (39 snakes), while only five (5) snakes which parasites
 116 spread to their tracheas.

117

118 **Table 1.** Prevalence of pentastomiasis in *Ptyas mucosa* based on site and the number of parasites.

Sites	Positive (%)	Number of parasites
Lungs only	57% (34 of 60)	2-9 with an average of 5 Pentastomida
Trachea only	0% (0 of 60)	-
Lungs and Trachea	8% (5 of 60)	7-9 with an average of 8 Pentastomida
Total	65% (39 of 60)	204 pentastomida

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Figure 1. Pentastomid infestation in the lungs of *Ptyas mucosa*. The white arrows show areas with raised lesions around the attachment of pentastomids to the tissue. The black arrows show female pentastomids, and the red arrow shows male pentastomids.

This is the first study to determine the prevalence of pentastomiasis in *Ptyas mucosa* in Sidoarjo, East Java, Indonesia.

Our results showed that pentastomids were present in 39 of 60 (65%) snakes (Table 1). This finding shows the prevalence of pentastomiasis in *P. mucosa* was quite high. This study's prevalence is similar to pentastomiasis in Brazil that 8 of 15 or 53.33% of snakes were positively infected with pentastomida. The food consumed by the snakes probably caused the high prevalence in this research. The type of food is very influential on the prevalence of infection and the type of pentastomida that infected the snakes (Almeida et al. 2007). *P. mucosa* snakes usually feed on rats, frogs, toads, lizards, and even smaller non-venomous and smaller snakes (Sidik 2006). In addition, according to Esslinger (1962), rats, frogs, toads, lizards, and even non-venomous snakes can act as the intermediate hosts of pentastomida.

Identification of pentastomida

The characteristics of pentastomid found in this study can be seen in Table 2 and Figure 2. Pentastomids have a long cylindrical body with a larger head, a flat shape on the ventral, and a tapered tail or posterior. Their body was soft and whitish with a colorless cuticle, which tended to be transparent to make the internal organs visible. There were annuli throughout their body up to the posterior. There are two pairs of hooks (a total of four) around the head that attach to the host's lung tissue, and it has an ovoid-shaped mouth between the two pairs of hooks.

Table 2. The characteristics of pentastomida compared with *Kiricephalus pattoni* by Riley and Self (1980) and Keegan (1943).

Parameters	The current study		British Museum collection (Riley and Self 1980)		Keegan 1943
	Females	Males	Females	Males	
Total number of parasites	183	21	17	2	-
Body length	72-125 mm (\bar{X} 94.5 mm)	17-28.5 mm (\bar{X} 23.75 mm)	83-137 mm (\bar{X} 100 mm)	28-29 mm (\bar{X} 28.5 mm)	-
Number of annuli	29-41 (\bar{X} 31.6)	28-35 (\bar{X} 31)	34-38 (\bar{X} 36.5)	31-32 (\bar{X} 31.5)	-

Diameter of cephalothorax	4-5.5 mm (\bar{X} 4.75 mm)	2-2.5 mm (\bar{X} 2 mm)	4 mm	-	-
Neck	2-3 mm (\bar{X} 2.5 mm)	-	2 mm	-	-
Diameter of abdomen	3.5-5 mm (\bar{X} 4.2 mm)	1.8-2.2 mm (\bar{X} 2 mm)	4 mm	-	-
Hook parameter:			-	-	-
a. AC					
b. CB	0.23-0.55 mm	0.22-0.35 mm			
c. AD	0.37-0.69 mm 0.47-0.88 mm	0.29-0.35 mm 0.29-0.45 mm			
Mouth:			-	-	-
a. Length	0.16-29 mm	0.14-0.38 mm			
b. Width	0.12-0.24 mm	0.12-0.18 mm			
Egg:		-	-	-	-
a. Length	114-130 μ m (\bar{X} 118 μ m)				125-154 μ m (\bar{X} 133 μ m)
b. Width	108-124 μ m (\bar{X} 116 μ m)				106-143 μ m (\bar{X} 123 μ m)

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Figure 2. Differences in size between females (top) and males (below).

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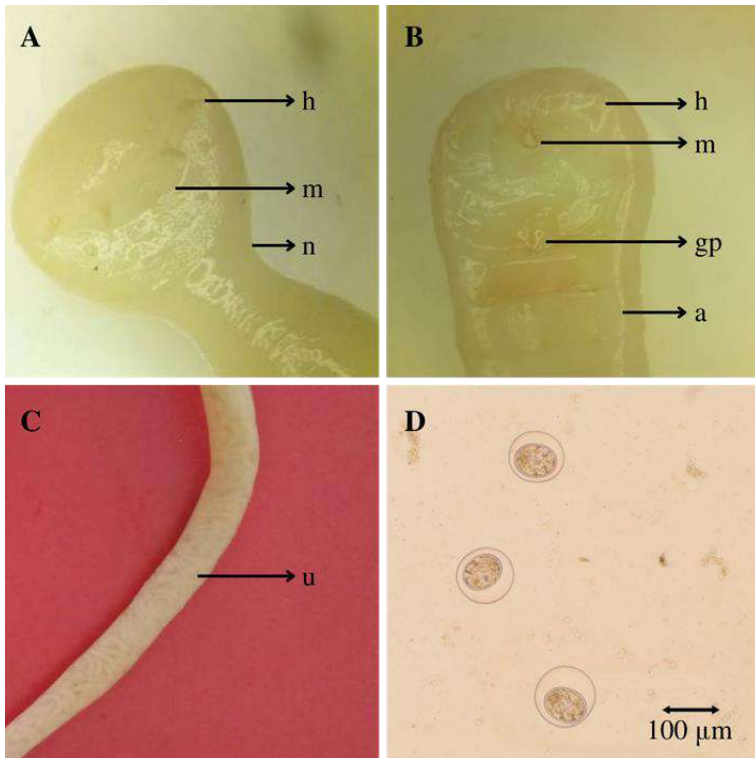
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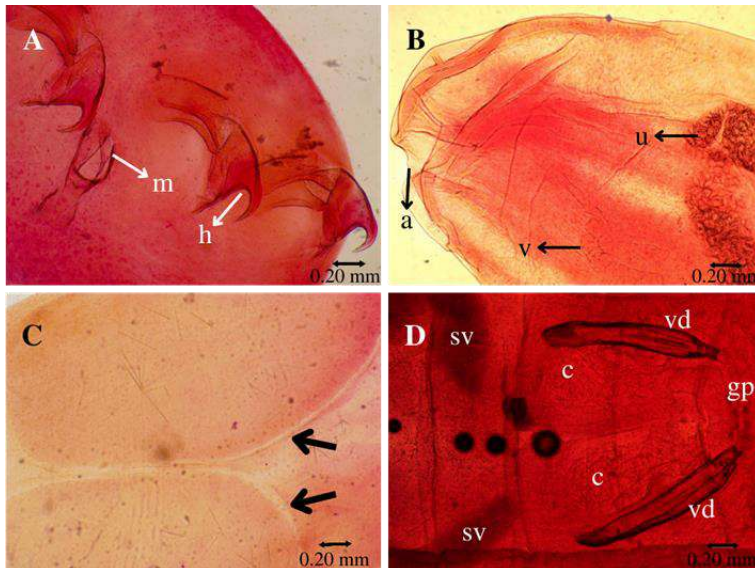
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Both sexes, adult males and female pentastomids were found in this research (Figures 1 and 2). The number of male pentastomid was less (21 heads) than that of females (183 heads) Table 2. Table 2 also describes the size of each pentastomid. The female pentastomids had longer and larger bodies than the males (Figures 1 and 2). The length of the female bodies was 72-125 mm, with an average of 94.5 mm. Their abdominal diameter was 3.5-5 mm, averaging 4.2 mm. Furthermore, the anterior section or cephalothorax of the females was globular, with a neck separating their head and abdomen (Figure 3A). The average length and width of the female mouths were 0.24 mm and 0.16 mm. Furthermore, the results of the microscopy examination using Carmine staining showed that the female pentastomid had two spermathecae on the anterior part. That connects directly to the uterus that contains the egg (Figures 3C, 4B, and 4C). In addition, the vagina was in the posterior body and around the anus (Figure 4B).



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157 **Figure 3.** Pentastomida without staining. (A) The anterior section of the female. (B) The anterior section of the male. (C) Female body.
158 (D) Pentastomid's eggs. h: hook, m: mouth, n: neck, gp: genital pore, a: first annuli, u: uterus.
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160 On the other hand, the length of the males' bodies was 17-28.5 mm, with an average of 23.75 mm. Their abdominal
161 diameter was 1.8-2.2 mm, averaging 2 mm. The average length and width of the mouth were 0.23 and 0.14 mm,
162 respectively. The male body was simple and straight without any curves in the neck (Figure 3B). The genital pore was
163 identified in the first annulus, and there was a pair of seminal vesicles, vas deferens, and cirrus (4D).
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165
166 **Figure 4.** Carmine-stained pentastomida. (A) The anterior section. (B) The posterior section of the female. (C) A pair of female
167 spermatheca. (D) Male reproductive organs. m: mouth, h: hook, a: anus, v: vagina, u: uterus, sv: seminal vesicle, c: cirrus, vd: vas
168 deferens.
169

170 The eggs were oval and consisted of two layers of membrane that clearly could be distinguished. The first layer (outer
171 membrane) was thin, while the second layer (inner membrane) was thick and yellowish. There was fluid-filled space
172 between the first and second layers. The length of the eggs was 114-130 μm with an average of 118 μm, and the width was
173 108-124 μm with an average of 116 μm (Figure 3D).

174 Pentastomida was mostly found in the lungs, and the parasites also spread to the trachea in five snakes. This finding
175 was similar to research by Riley and Self (1980) that revealed that adult *Kiricephalus pattoni* (one of the pentastomida

176 species) were only found in the lungs of *P. mucosa* snakes. In our observation, pentastomids that spread to the trachea
177 were females. According to Sundar et al. (2015), the adult pentastomids with mature eggs are expelled from the trachea
178 and eliminated from the definitive host by oral expulsion or may also be swallowed. That results in shed eggs in the feces,
179 which another intermediate mammal host will ingest to continue their life cycles.

180 The number of male pentastomids was fewer than the number of female ones. This case was also experienced by John
181 and Nadakal (1988) and Riley and Self (1980), who only found a few males compared the female. This study result
182 follows Almeida et al. (2006), whose number of pentastomids and their species is unknown in biology.

183 The taxonomy of pentastomida is still controversial. Moreover, it has been known through molecular data that
184 pentastomida are closely related to crustaceans and belong to the class Maxillopoda, sub-phylum Crustacea, and the
185 phylum Arthropoda (Paré 2008). Based on the egg size (Almeida et al. 2007); the female head form that globular with a
186 neck separating the head from its body (Paré 2008); and the size of the body length, the number of the annuli, the diameter
187 of the cephalothorax, neck, abdominal diameter, head, and body shape (Riley and Self 1979; Riley and Self 1980);
188 pentastomida was classified as *K. pattoni*. *K. pattoni* can be classified as Porocephalida (Paré 2008). In addition, according
189 to Christoffersen and De Assis (2013; 2015), pentastomid in *P. mucosa* snakes are classified into *K. pattoni*.

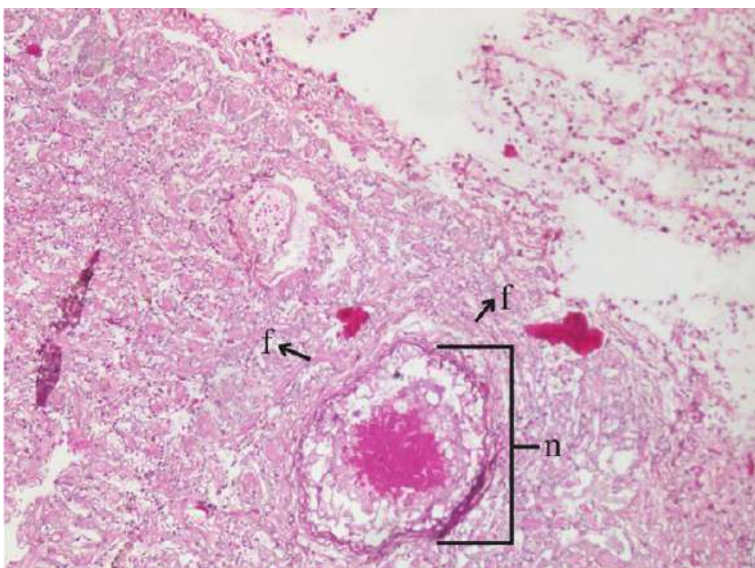
190 *K. pattoni* has a long annulated body, a tapered tail, an anal canal, two pairs of hooks or four hooks on its head, and a
191 small ovoid-shaped mouth (Riley and Self, 1979). The female *K. pattoni* has a larger body than the male one (Paré 2008).
192 Female *K. pattoni* also has a spiral body with a larger head than its body, and a curve of the neck, whereas male one has a
193 straight body without a curve on the neck (Riley and Self 1979; Riley and Self 1980; John and Nadakal 1988; Almeida et
194 al. 2007). The spiral body in females is likely to facilitate their movement into the lung tissue of the definitive hosts (John
195 and Nadakal 1988).

196 The egg morphology is similar to the eggs of *Porocephalus crotali* described by Sundar et al. (2015). These eggs were
197 composed of two-layer membranes, of which the outer one was thin and the inner membrane thick, and there was a fluid-
198 filled space between those two membranes. But the average diameter of the eggs measured in this investigation is smaller
199 (118 x 116 µm) than the eggs of *P. crotali* (144 µm), as described by Sundar et al. (2015).

201 **Histopathological changes in the lungs of *Ptyas mucosa***

202 Squamates typically have two lungs; however, the left lung in most snakes is greatly reduced, never exceeding 85% of
203 the right lung's size or absence (Funk and Bogan 2019). The right lung usually runs from near the heart to the cranial to the
204 right kidney (Funk and Bogan 2019). The cranial portion of a lung is vascularized and functions primarily as a gas
205 exchanger (vascular lung), whereas the caudal primarily functions as an air sac (saccular lung). The vascular lung's wall
206 comprises honeycombed units called faveoli, allowing gas exchange (Funk and Bogan 2019). The faveoli in the snake lung
207 parenchyma are lined microscopically by squamous epithelial cells (type I cells). In addition to a lesser degree, by cuboidal
208 epithelial cells (type II) (Jacobson et al. 2021).

209 In this study, a nymphal stage of the parasite was found in the pulmonary tissue infected with pentastomida. The
210 parasite is surrounded by some connective tissue (Figure 5). Finding the stages of the nymph in the snake's histological
211 lung and the parasite's stages in the macroscopic lung organ means that the *P. mucosa* snake may act as an intermediate
212 and final host of *K. pattoni*. Within the lung cavities of the definitive host, the nymphs develop into adults (Sundar et al.
213 2015). In this study, pentastomida infection in the lungs only caused a mild histological reaction in the form of fibrosis in
214 the area around the parasite.
215



216 **Figure 5.** Histological microscopic picture of nymph-infected Pulmo from pentastomida showed the presence of fibroblast around the
217 area of infection. f: fibroblast, n: the body of the nymph stage pentastomida.
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Pentastomida has an indirect life cycle that typically utilizes vertebrate hosts. Consequently, these species rely on reptiles as their definitive host. The adults are usually found in their lungs (Wellehan and Walden 2019). Pentastomida parasites migrate extensively through the visceral before settling in the lungs and, less typically, the trachea and nasal passageways. Pentastomida can produce localized lesions, while molting can elicit antigenic reactions. Adults feed on lung tissue fluids and pass embryonated eggs, which are coughed up through the oral cavity, swallowed, and passed in the feces (Comolli and Divers 2021). In squamates, the pulmonary lumen is large, and the tracheal diameter may be smaller than that of the pentastomids. That poses a risk of respiratory obstruction by the adult pentastomids. However, the greatest pathological changes are seen in the intermediate host, where they may become embedded in tissues (Wellehan and Walden 2019).

Pentastomiasis in reptiles, especially snakes, occurs subclinically. Although the infection is large, death is usually associated with secondary septicemia and pneumonia (Tappe and Büttner 2009). Because pentastomids attach to the lung tissue, they can cause perforation, hemorrhaging, and serious inflammation in the host (Fernando and Fernando 2014). In a Common Garter Snake (*Thamnophis sirtalis*), lung lesions were associated with one of the pentastomida species, *Kiricephalus coarctatus*, where the embedded parasite had gotten into the hypaxial muscle. Infections with *Armillifer armillatus* have also been linked to parasitic pneumonia in *Gaboon vipers*. Pentastomids occluding the trachea have been implicated in snake deaths. Bacterial infections could develop due to lung damage. For example, there are four out of nine *Boa constrictors* with pulmonary damage from *Porocephalus dominicana* infection perished due to bacteremia and pericarditis (Walden et al. 2021).

Bigger implications regarding possible impacts on human health may be anticipated based on the histopathological changes found in this study. Humans are the final hosts of pentastomids, which can be contracted from improperly cooked meat from infected snakes or drinking water contaminated with pentastomida eggs. In addition, humans can get the infection when handling and harvesting snakeskin or having direct contact with the mouth of a pet snake (Latif et al. 2016). The pathological consequences of the clinical symptoms experienced by infected humans may be the same or even more severe. That depends on the number of parasite eggs ingested, the duration from the larvae migrating to various organs to maturity and reproduction, and how severe the parasite infestation is in the human viscera.

Personal hygiene measures are required to prevent human infections. For example, avoid drinking river water directly or boiling the water before drinking. In addition, authorities should provide health education to warn people about eating undercooked snake meat, the possibility of transmission after handling snakes, and avoiding contact with snake excretions.

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CONCLUSION

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The prevalence of pentastomiasis in slaughtered *Ptyas mucosa* in Sidoarjo was 65% (39 of 60 snakes). The pentastomids that infect these snakes are classified as *Kiricephalus pattoni*. The pulmonary histological findings only show a light reaction in the form of mild fibrosis.

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First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia

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Abstract. Plumeriastuti H, Pranashinta GT, Suwanti LT, Kusnoto, Proboningrat A. 2023. First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia. *Biodiversitas* 24: xxxx. Currently, reptiles, especially snakes, are increasingly popular as pets worldwide and are often consumed by several communities in Indonesia. This trend needs attention from a public health perspective because it can potentially cause zoonotic diseases, including parasitic disease and pentastomiasis. This study aimed to determine: the prevalence of pentastomiasis, identify the parasite and observe the pulmonary histopathology in Oriental rat snakes (*Ptyas mucosa* Linnaeus 1758) collected in Tulangan, Sidoarjo, Indonesia. The methods used were calculating the prevalence of pentastomiasis, semichen-acetic carmine staining, and identifying parasites and eggs based on references as a comparison. Furthermore, histopathological preparation and hematoxylin-eosin staining were performed on the lungs infected with the parasite to observe the microscopic lesions. The results showed that 65% of the snakes had pentastomiasis, and the parasites were called *Kirichephalus pattoni* Stephens 1908. Furthermore, a microscopical examination revealed mild fibrosis in the pulmonary area due to pentastomid infestation. This study is the first reported pentastomida in *P. mucosa* in Sidoarjo, Indonesia, showing that snakes caught and commonly consumed were infected with zoonotic pentastomic.

Keywords: Colubrid snake, parasitic pneumonia, tongue worms, zoonotic parasitoses

INTRODUCTION

Exotic animals such as reptiles, especially various snakes, are now popular as pets worldwide (Okulewicz et al. 2014). However, they are also concerned with the increasing animal welfare and health issues through the animal trade in the market (Yudhana et al. 2021). Some species of snake are bred in captivity, while others are caught in the wild and sold as pets or bushmeat for consumption (Hardi et al. 2017; Yudhana et al. 2021).

Ptyas mucosa Linnaeus 1758 is a well-known snake species in Indonesia (Pranashinta et al. 2017). This type of snake, known as the Oriental rat snake and Indian rat snake, belongs to the Colubridae family which is widely distributed throughout East, South, and Southeast Asia (Auliya 2010; Muliya and Bhat 2016; Yuan et al. 2022). *P. mucosa* is a diurnal and semi-arboreal snake species that live on forest floors, agricultural fields, wet ground, and near human habitats. It feeds on frogs, rats, and other small animals (Chittora et al. 2021).

The meat of *P. mucosa* is used in the illegal trade to China (Auliya 2010). The meat and internal organs are used as fresh or frozen food, the bile and blood are used in traditional medicine, and the skin is exported to several European countries as raw materials for the leather industry (Pranashinta et al. 2017). Although illegal, most rural communities in Indonesia trade and consume the meat and

bile of *P. mucosa* for some reasons (Auliya 2010; Pranashinta et al. 2017).

Wildlife meat, however, poses a threat of transmitting infection with novel and exotic pathogens to human hosts (Hardi et al. 2017). Then new zoonotic diseases are likely to emerge consequently. For instance, Bats (Chiroptera) are important reservoirs of several emerging diseases, including Ebola virus disease (EVD), Severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS), as well as the current pandemic COVID-19 caused by SARS Coronavirus 2 (Irving et al. 2021). Moreover, many parasites also cause emerging zoonotic diseases, resulting in a significant public health burden, particularly in tropical countries (Alasil and Abdullah 2019).

Several parasites in confined reptiles spend a portion of their life cycle in the respiratory system. Most respiratory involvement is asymptomatic and mild, generating mostly localized inflammation except for excessive parasite loads. However, severe disease can result from high burdens or unusual host-parasite interactions, frequently worsened by secondary infections (Comolli 2021). Reptile-borne pentastomiasis is an unusual tropical disease neglected and under-researched in Asia, Africa, and South America (Sulyok et al. 2014).

Pentastomiasis is a parasitic zoonosis caused by infection with pentastomic, belonging to the subclass

Pentastomida, under the phylum Arthropoda (Chittora et al. 2021; Tongtako et al. 2013; WHA 2019). Pentastomiasis is also known as tongue worms since the adult phase of the genus *Linguatula* has similarities with mammalian tongues (Paré 2008). Adult parasites are usually found in the respiratory tract of reptiles, birds, and mammals, including humans, while their larval forms are in the internal organs of insects, mammals, and amphibians (Paré 2008; Chen et al. 2010; Kelehear et al. 2014). Some zoonotic Pentastomida in snakes is *Armillifer* and *Porocephalus crotali* Humboldt 1812 (Rataj et al. 2011). Humans can be infected with pentastomids after they consume raw snake meat, have direct contact with the snake, drink water contaminated with parasitic eggs, or are contaminated through the feces (Kelehear et al. 2014; Tappe et al. 2016; Mendoza-Roldan et al. 2020).

Pentastomiasis in snakes have been reported in many countries, such as poisonous Terciopelo (*Bothrops asper* Garman 1883) in Costa Rica (Alvarado et al. 2015), Royal python (*Python regius* Shaw 1802) snakes in Nigeria (Ayinmode et al. 2010), Rat snakes (*P. mucosa*) in Sri Lanka and India (Fernando and Fernando 2014; Sundar et al. 2015), wild snakes in Australia (Kelehear et al. 2014), various species of snakes in Slovenia and Pakistan (Rataj et al. 2011), Chinese moccasin (*Agkistrodon acutus*) and Indian python (*Python molurus* Linnaeus 1758) in China (Chen et al. 2010), and Totonacan rattlesnake (*Crotalus totonacus* Gloyd & Kauffeld 1940) in Mexico (Tepos-Ramírez et al. 2022).

Moreover, to our best knowledge, snakes in Indonesia have no reports of pentastomid infections. Therefore, this study reported the prevalence and identified pentastomida and the pulmonary histopathological profiles in Oriental rat snakes (*P. mucosa*) in Sidoarjo Sub-district, East Java, Indonesia.

MATERIALS AND METHODS

Ethical approval

The Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga (number 678-KE) reviewed and approved this study.

Parasite collection

The sample used comes from the Oriental rat snake (*P. mucosa*), which is often caught and sold for its meat and skin through collectors. The collection was conducted at a snake slaughterhouse in Tulangan Sub-district, Sidoarjo District, East Java Province, Indonesia. The presence of pentastomid parasites was observed in the respiratory tract of 60 slaughtered snakes. Pentastomides were taken and cleaned with distilled water, then stored in sterile plastic pots. The lungs of the snakes infected with the parasite were also isolated for histopathological preparation.

Prevalence calculation

The following formula calculated the prevalence of pentastomiasis in snake samples:

$$\text{Prevalence (\%)} = \frac{\text{(The number of snakes infected with pentastomids)}}{\text{(Total sample of snakes)}} \times 100\%$$

Semichen-acetic carmine staining

The collected pentastomids were stained with the Semichen-Acetic Carmine technique (Kuhlmann 2006). Adult pentastomids were fixed on object glasses and soaked in 5% glycerin alcohol for 24 hours. Then they were soaked in 70% alcohol for five minutes. Subsequently, the object glasses with pentastomids were placed in Carmine alcohol solution (1:2) for eight hours. Next, the pentastomids were soaked in acid alcohol for 10 minutes and moved to base alcohol for 20 minutes. Furthermore, graded dehydration was carried out with 70%, 85%, and 95% alcohol for five minutes. The mounting step was done using Hung's I for 20 minutes. Finally, pentastomids were taken and placed on new clean object glasses. The pentastomids were dripped with Hung's II solutions and then covered with a cover glass.

Parasite identification

Characteristics of the head and body shape and body size of the parasite were identified based on Riley and Self (1980), Almedia et al. (2006), and Christoffersen and De Assis (2013; 2015). Identification of parasite egg size was carried out according to Keegan (1943).

The fresh parasite's length was measured from the anterior edge to the posterior end. The head was observed under a stereo microscope and documented with a Canon Digital Single-Lens Reflex (DSLR) camera. Furthermore, stained parasites were observed using a light microscope with 40-100× magnification and documented using Miconos Optilab. Some female parasites were incubated in PBS overnight at 37°C to collect the eggs. The supernatants were centrifuged at 1,500 rpm for 10 minutes, and the pellet was dripped onto a glass object and observed using a light microscope with 100× magnification. Those eggs were then measured using Miconos Optilab.

Histopathological preparations

The lungs of snakes with pentastomid infestation were fixed in 10% formalin. Then graded dehydration was carried out with serial alcohol concentrations (70%, 80%, 90%, 96%, and absolute) and clearing using xylol. Furthermore, paraffin embedding was performed to obtain a solid block of tissue. The tissue blocks were sliced (3 micrometers thick) using a microtome, mounted on an object glass, and stained using Hematoxylin and Eosin. Microscopic examination was performed using Nikon Eclipse Ci.

RESULTS AND DISCUSSIONS

The prevalence of pentastomiasis in *P. mucosa* snakes

Thirty-nine (or 65%) of the respiratory tract of 60 samples were positively infected with adult pentastomids; a total of 204 pentastomids samples were collected (Table 1). Pentastomida was found to be strongly attached to the trachea and lungs of snakes (Figure 1). Some pentastomids

even buried their heads in the lungs of snakes. The parasites of all snakes positively infected with pentastomids were found in the lungs (39 snakes), while only five (5) snakes which parasites spread to their tracheas.

This is the first study to determine the prevalence of pentastomiasis in *P. mucosa* in Sidoarjo, East Java, Indonesia. Our results showed that pentastomids were present in 39 of 60 (65%) snakes (Table 1). This finding shows the prevalence of pentastomiasis in *P. mucosa* was quite high. This study's prevalence is similar to pentastomiasis in Brazil that 8 of 15 or 53.33% of snakes were positively infected with pentastomida. The food consumed by the snakes probably caused the high prevalence in this research. The type of food is very influential on the prevalence of infection and the type of pentastomida that infected the snakes (Almeida et al. 2007). *P. mucosa* snakes usually feed on rats, frogs, toads, lizards, and even smaller non-venomous and smaller snakes (Sidik 2006). In addition, according to Esslinger (1962), rats, frogs, toads, lizards, and even non-venomous snakes can act as the intermediate hosts of pentastomida.

Identification of pentastomida

The characteristics of pentastomid found in this study can be seen in Table 2 and Figure 2. Pentastomids have a long cylindrical body with a larger head, a flat shape on the ventral, and a tapered tail or posterior. Their body was soft and whitish with a colorless cuticle, which tended to be transparent to make the internal organs visible. There were annuli throughout their body up to the posterior. There are two pairs of hooks (a total of four) around the head that attach to the host's lung tissue, and it has an ovoid-shaped mouth between the two pairs of hooks.

Both sexes, adult males and female pentastomids were found in this research (Figures 1 and 2). The number of male pentastomid was less (21 heads) than that of females (183 heads) Table 2. Table 2 also describes the size of each pentastomid. The female pentastomids had longer and larger bodies than the males (Figures 1 and 2). The length of the female bodies was 72-125 mm, with an average of 94.5 mm. Their abdominal diameter was 3.5-5 mm, averaging 4.2 mm. Furthermore, the anterior section or cephalothorax of the females was globular, with a neck separating their head and abdomen (Figure 3A). The average length and width of the female mouths were 0.24 mm and 0.16 mm. Furthermore, the results of the microscopy examination using Carmine staining showed that the female pentastomid had two spermathecae on the anterior part. That connects directly to the uterus that contains the egg (Figures 3C, 4B, and 4C). In addition, the vagina was in the posterior body and around the anus (Figure 4B).

On the other hand, the length of the males' bodies was 17-28.5 mm, with an average of 23.75 mm. Their abdominal diameter was 1.8-2.2 mm, averaging 2 mm. The average length and width of the mouth were 0.23 and 0.14 mm, respectively. The male body was simple and straight without any curves in the neck (Figure 3B). The genital

pore was identified in the first annulus, and there was a pair of seminal vesicles, vas deferens, and cirrus (4D).

The eggs were oval and consisted of two layers of membrane that clearly could be distinguished. The first layer (outer membrane) was thin, while the second layer (inner membrane) was thick and yellowish. There was fluid-filled space between the first and second layers. The length of the eggs was 114-130 μm with an average of 118 μm , and the width was 108-124 μm with an average of 116 μm (Figure 3D).

Table 1. Prevalence of pentastomiasis in *P. mucosa* based on site and the number of parasites.

Sites	Positive (%)	Number of parasites
Lungs only	57% (34 of 60)	2-9 with an average of 5 Pentastomida
Trachea only	0% (0 of 60)	-
Lungs and Trachea	8% (5 of 60)	7-9 with an average of 8 Pentastomida
Total	65% (39 of 60)	204 pentastomida



Figure 1. Pentastomid infestation in the lungs of *P. mucosa*. The white arrows show areas with raised lesions around the attachment of pentastomids to the tissue. The black arrows show female pentastomids, and the red arrow shows male pentastomids.

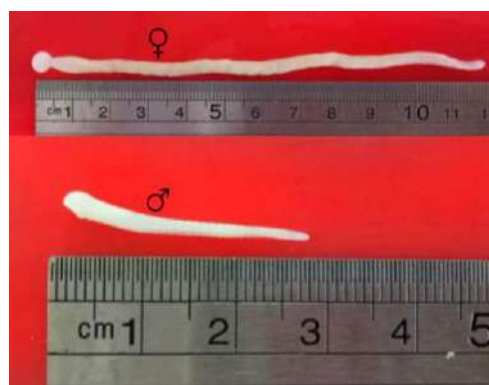


Figure 2. Differences in size between females (top) and males (below).

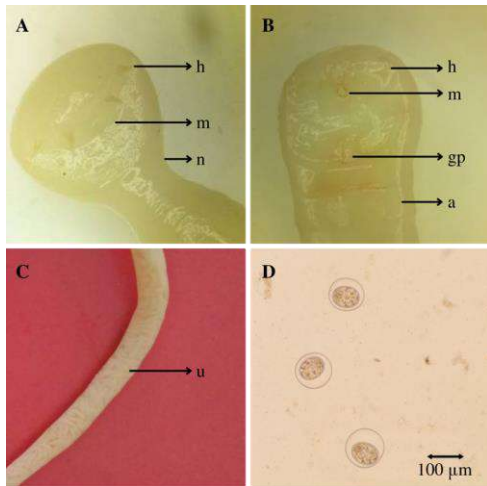


Figure 3. Pentastomida without staining. (A) The anterior section of the female; (B) The anterior section of the male; (C) Female body; (D) Pentastomid's eggs. h: hook, m: mouth, n: neck, gp: genital pore, a: first annuli, u: uterus.

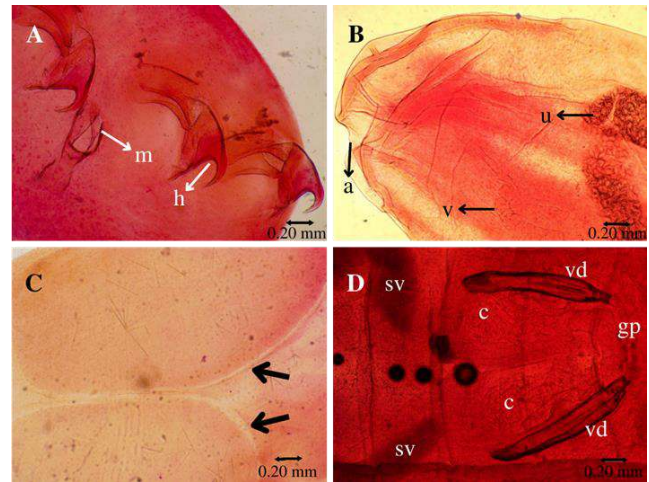


Figure 4. Carmine-stained pentastomida. (A) The anterior section; (B) The posterior section of the female; (C) A pair of female spermatheca; (D) Male reproductive organs. m: mouth, h: hook, a: anus, v: vagina, u: uterus, sv: seminal vesicle, c: cirrus, vd: vas deferens.

Table 2. The characteristics of pentastomida compared with *Kirichephalus pattoni* Stephens 1908 by Riley and Self (1980) and Keegan (1943).

Parameters	The current study		British Museum collection (Riley and Self 1980)		Keegan 1943
	Females	Males	Females	Males	
Total number of parasites	183	21	17	2	-
Body length	72-125 mm (\bar{X} 94.5 mm)	17-28.5 mm (\bar{X} 23.75 mm)	83-137 mm (\bar{X} 100 mm)	28-29 mm (\bar{X} 28.5 mm)	-
Number of annuli	29-41 (\bar{X} 31.6)	28-35 (\bar{X} 31)	34-38 (\bar{X} 36.5)	31-32 (\bar{X} 31.5)	-
Diameter of cephalothorax	4-5.5 mm (\bar{X} 4.75 mm)	2-2.5 mm (\bar{X} 2 mm)	4 mm	-	-
Neck	2-3 mm (\bar{X} 2.5 mm)	-	2 mm	-	-
Diameter of abdomen	3.5-5 mm (\bar{X} 4.2 mm)	1.8-2.2 mm (\bar{X} 2 mm)	4 mm	-	-
Hook parameter:					
AC			-	-	-
CB	0.23-0.55 mm	0.22-0.35 mm			
AD	0.37-0.69 mm	0.29-0.35 mm			
	0.47-0.88 mm	0.29-0.45 mm			
Mouth:					
Length	0.16-29 mm	0.14-0.38 mm	-	-	-
Width	0.12-0.24 mm	0.12-0.18 mm			
Egg:					
Length	114-130 µm (\bar{X} 118 µm)	-	-	-	125-154 µm (\bar{X} 133 µm)
Width	108-124 µm (\bar{X} 116 µm)				106-143 µm (\bar{X} 123 µm)

Pentastomida was mostly found in the lungs, and the parasites also spread to the trachea in five snakes. This finding was similar to research by Riley and Self (1980) that revealed that adult *K. pattoni* (one of the pentastomida species) were only found in the lungs of *P. mucosa* snakes. In our observation, pentastomids that spread to the trachea were females. According to Sundar et al. (2015), the adult

pentastomids with mature eggs are expelled from the trachea and eliminated from the definitive host by oral expulsion or may also be swallowed. That results in shed eggs in the feces, which another intermediate mammal host will ingest to continue their life cycles.

The number of male pentastomids was fewer than the number of female ones. This case was also experienced by

John and Nadakal (1988) and Riley and Self (1980), who only found a few males compared the female. This study result follows Almeida et al. (2006), whose number of pentastomids and their species is unknown in biology

The taxonomy of pentastomida is still controversial. Moreover, it has been known through molecular data that pentastomida are closely related to crustaceans and belong to the class Maxillopoda, sub-phylum Crustacea, and the phylum Arthropoda (Paré 2008). Based on the egg size (Almeida et al. 2007); the female head form that globular with a neck separating the head from its body (Paré 2008); and the size of the body length, the number of the annuli, the diameter of the cephalothorax, neck, abdominal diameter, head, and body shape (Riley and Self 1979; Riley and Self 1980); pentastomida was classified as *K. pattoni*. *K. pattoni* can be classified as Porochepalida (Paré 2008). In addition, according to Christoffersen and De Assis (2013; 2015), pentastomid in *P. mucosa* snakes are classified into *K. pattoni*.

K. pattoni has a long annulated body, a tapered tail, an anal canal, two pairs of hooks or four hooks on its head, and a small ovoid-shaped mouth (Riley and Self 1979). The female *K. pattoni* has a larger body than the male one (Paré 2008). Female *K. pattoni* also has a spiral body with a larger head than its body, and a curve of the neck, whereas male one has a straight body without a curve on the neck (Riley and Self 1979; Riley and Self 1980; John and Nadakal 1988; Almeida et al. 2007). The spiral body in females is likely to facilitate their movement into the lung tissue of the definitive hosts (John and Nadakal 1988).

The egg morphology is similar to the eggs of *P. crotali* described by Sundar et al. (2015). These eggs were composed of two-layer membranes, of which the outer one was thin and the inner membrane thick, and there was a fluid-filled space between those two membranes. But the average diameter of the eggs measured in this investigation is smaller (118x116 µm) than the eggs of *P. crotali* (144 µm), as described by Sundar et al. (2015).

Histopathological changes in the lungs of *P. mucosa*

Squamates typically have two lungs; however, the left lung in most snakes is greatly reduced, never exceeding 85% of the right lung's size or absence (Funk and Bogan 2019). The right lung usually runs from near the heart to the cranial to the right kidney (Funk and Bogan 2019). The cranial portion of a lung is vascularized and functions primarily as a gas exchanger (vascular lung), whereas the caudal primarily functions as an air sac (saccular lung). The vascular lung's wall comprises honeycombed units called faveoli, allowing gas exchange (Funk and Bogan 2019). The faveoli in the snake lung parenchyma are lined microscopically by squamous epithelial cells (type I cells). In addition to a lesser degree, by cuboidal epithelial cells (type II) (Jacobson et al. 2021).

In this study, a nymphal stage of the parasite was found in the pulmonary tissue infected with pentastomida. The parasite is surrounded by some connective tissue (Figure 5). Finding the stages of the nymph in the snake's histological lung and the parasite's stages in the macroscopic lung organ means that the *P. mucosa* snake

may act as an intermediate and final host of *K. pattoni*. Within the lung cavities of the definitive host, the nymphs develop into adults (Sundar et al. 2015). In this study, pentastomida infection in the lungs only caused a mild histological reaction in the form of fibrosis in the area around the parasite.

Pentastomida has an indirect life cycle that typically utilizes vertebrate hosts. Consequently, these species rely on reptiles as their definitive host. The adults are usually found in their lungs (Wellehan and Walden 2019). Pentastomida parasites migrate extensively through the visceral before settling in the lungs and, less typically, the trachea and nasal passageways. Pentastomida can produce localized lesions, while molting can elicit antigenic reactions. Adults feed on lung tissue fluids and pass embryonated eggs, which are coughed up through the oral cavity, swallowed, and passed in the feces (Comolli and Divers 2021). In squamates, the pulmonary lumen is large, and the tracheal diameter may be smaller than that of the pentastomids. That poses a risk of respiratory obstruction by the adult pentastomids. However, the greatest pathological changes are seen in the intermediate host, where they may become embedded in tissues (Wellehan and Walden 2019).

Pentastomiasis in reptiles, especially snakes, occurs subclinically. Although the infection is large, death is usually associated with secondary septicemia and pneumonia (Tappe and Büttner 2009). Because pentastomids attach to the lung tissue, they can cause perforation, hemorrhaging, and serious inflammation in the host (Fernando and Fernando 2014). In a Common Garter Snake (*Thamnophis sirtalis* Linnaeus 1758), lung lesions were associated with one of the pentastomida species, *Kiricephalus coarctatus* Diesing 1850, where the embedded parasite had gotten into the hypaxial muscle. Infections with *Armillifer armillatus* Wyman 1845 have also been linked to parasitic pneumonia in gaboon vipers. Pentastomids occluding the trachea have been implicated in snake deaths. Bacterial infections could develop due to lung damage. For example, there are four out of nine *Boa constrictors* with pulmonary damage from *Porocephalus dominicana* Riley & Walters 1980 infection perished due to bacteremia and pericarditis (Walden et al. 2021).

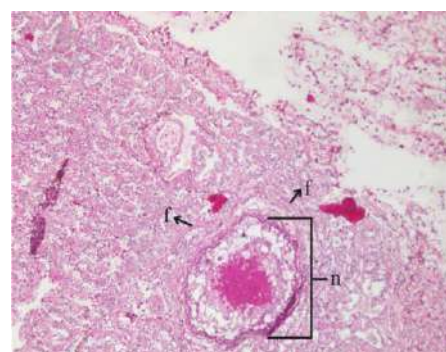


Figure 5. Histological microscopic picture of nymph-infected pulmo from pentastomida showed the presence of fibroblast around the area of infection. f: fibroblast, n: the body of the nymph stage pentastomida.

Bigger implications regarding possible impacts on human health may be anticipated based on the histopathological changes found in this study. Humans are the final hosts of pentastomids, which can be contracted from improperly cooked meat from infected snakes or drinking water contaminated with pentastomida eggs. In addition, humans can get the infection when handling and harvesting snakeskin or having direct contact with the mouth of a pet snake (Latif et al. 2016). The pathological consequences of the clinical symptoms experienced by infected humans may be the same or even more severe. That depends on the number of parasite eggs ingested, the duration from the larvae migrating to various organs to maturity and reproduction, and how severe the parasite infestation is in the human viscera.

Personal hygiene measures are required to prevent human infections. For example, avoid drinking river water directly or boiling the water before drinking. In addition, authorities should provide health education to warn people about eating undercooked snake meat, the possibility of transmission after handling snakes, and avoiding contact with snake excretions.

In conclusion the prevalence of pentastomiasis in slaughtered *P. mucosa* in Sidoarjo was 65% (39 of 60 snakes). The pentastomids that infect these snakes are classified as *K. pattoni*. The pulmonary histological findings only show a light reaction in the form of mild fibrosis.

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First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia

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Abstract. Plumeriastuti H, Pranashinta GT, Suwanti LT, Proboningrat A, Kusnoto, ~~Proboningrat A~~. 2023. First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia. *Biodiversitas* 24: **xxxx**. Currently, reptiles, especially snakes, are increasingly popular as pets worldwide and are often consumed by several communities in Indonesia. This trend needs attention from a public health perspective because it can potentially cause zoonotic diseases, including parasitic disease ~~and such as~~ pentastomiasis. This study aimed to determine: the prevalence of pentastomiasis, identify the parasite and observe the pulmonary histopathology in Oriental ~~rat-Rat snakes-Snakes~~ (*Ptyas mucosa* Linnaeus 1758) collected in Tulangan, Sidoarjo, Indonesia. The methods used were calculating the prevalence of pentastomiasis, semichen-acetic carmine staining, and identifying parasites and eggs based on references as a comparison. Furthermore, histopathological preparation and hematoxylin-eosin staining were performed on the lungs infected with the parasite to observe the microscopic lesions. The results showed that 65% of the snakes had pentastomiasis, and the parasites were called *Kirichephalus pattoni* Stephens 1908. Furthermore, a microscopical examination revealed mild fibrosis in the pulmonary area due to pentastomid infestation. This study is the first reported pentastomida in *P. mucosa* in Sidoarjo, Indonesia, showing that snakes caught and commonly consumed were infected with zoonotic pentastomic.

Keywords: Colubrid snake, parasitic pneumonia, tongue worms, zoonotic parasitoses

INTRODUCTION

Exotic animals such as reptiles, especially various snakes, are now popular as pets worldwide (Okulewicz et al. 2014). However, they are also concerned with the increasing animal welfare and health issues through the animal trade in the market (Yudhana et al. 2021). Some species of snake are bred in captivity, while others are caught in the wild and sold as pets or bushmeat for consumption (Hardi et al. 2017; Yudhana et al. 2021).

Ptyas mucosa Linnaeus 1758 is a well-known snake species in Indonesia (Pranashinta et al. 2017). This type of snake, known as the Oriental ~~rat-Rat snake-Snake~~ and Indian ~~rat-Rat snakeSnake~~, belongs to the Colubridae family which is widely distributed throughout East, South, and Southeast Asia (Auliya 2010; Muliya and Bhat 2016; Yuan et al. 2022). *P. mucosa* is a diurnal and semi-arboreal snake species that live on forest floors, agricultural fields, wet ground, and near human habitats. It feeds on frogs, rats, and other small animals (Chittora et al. 2021).

The meat of *P. mucosa* is used in the illegal trade to China (Auliya 2010). The meat and internal organs are used as fresh or frozen food, the bile and blood are used in traditional medicine, and the skin is exported to several European countries as raw materials for the leather industry (Pranashinta et al. 2017). Although illegal, most rural communities in Indonesia trade and consume the meat and

bile of *P. mucosa* for some reasons (Auliya 2010; Pranashinta et al. 2017).

Wildlife meat, however, poses a threat of transmitting infection with novel and exotic pathogens to human hosts (Hardi et al. 2017). Then new zoonotic diseases are likely to emerge consequently. For instance, Bats (Chiroptera) are important reservoirs of several emerging diseases, including Ebola ~~virus-Virus disease-Disease~~ (EVD), Severe ~~acute-Acute respiratory-Respiratory syndrome-Syndrome~~ (SARS), and Middle East ~~respiratory-Respiratory syndrome Syndrome~~ (MERS), as well as the current pandemic COVID-19 caused by SARS Coronavirus 2 (Irving et al. 2021). Moreover, many parasites also cause emerging zoonotic diseases, resulting in a significant public health burden, particularly in tropical countries (Alasil and Abdullah 2019).

Several parasites in confined reptiles spend a portion of their life cycle in the respiratory system. Most respiratory involvement is asymptomatic and mild, generating mostly localized inflammation except for excessive parasite loads. However, severe disease can result from high burdens or unusual host-parasite interactions, frequently worsened by secondary infections (Comolli 2021). Reptile-borne pentastomiasis is an unusual tropical disease neglected and under-researched in Asia, Africa, and South America (Sulyok et al. 2014).

Pentastomiasis is a parasitic zoonosis caused by infection with pentastomic, belonging to the subclass Pentastomida, under the phylum Arthropoda (Chittora et al. 2021; Tongtako et al. 2013; WHA 2019). Pentastomiasis is also known as tongue worms since the adult phase of the genus *Linguatula* has similarities with mammalian tongues (Paré 2008). Adult parasites are usually found in the respiratory tract of reptiles, birds, and mammals, including humans, while their larval forms are in the internal organs of insects, mammals, and amphibians (Paré 2008; Chen et al. 2010; Kelehear et al. 2014). Some zoonotic Pentastomida in snakes is *Armillifer* and *Porocephalus crotali* Humboldt 1812 (Rataj et al. 2011). Humans can be infected with pentastomic after they consume raw snake meat, have direct contact with the snake, drink water contaminated with parasitic eggs, or are contaminated through the feces (Kelehear et al. 2014; Tappe et al. 2016; Mendoza-Roldan et al. 2020).

Pentastomiasis in snakes have been reported in many countries, such as poisonous Terciopelo (*Bothrops asper* Garman 1883) in Costa Rica (Alvarado et al. 2015), Royal python-Python Snakes (*Python regius* Shaw 1802) snakes in Nigeria (Ayinmode et al. 2010), Rat snakes-Snakes (*P. mucosa*) in Sri Lanka and India (Fernando and Fernando 2014; Sundar et al. 2015), wild snakes in Australia (Kelehear et al. 2014), various species of snakes in Slovenia and Pakistan (Rataj et al. 2011), Chinese moccasin-Moccasin (*Agkistrodon acutus*) and Indian python-Python (*Python molurus* Linnaeus 1758) in China (Chen et al. 2010), and Totonacan rattlesnake-Rattlesnake (*Crotalus totonacus* Gloyd & Kauffeld 1940) in Mexico (Tepos-Ramírez et al. 2022).

Moreover, to our best knowledge, snakes in Indonesia have no reports of pentastomic infections. Therefore, this study reported the prevalence and identified pentastomida and the pulmonary histopathological profiles in Oriental rat snakes—Snakes (*P. mucosa*) in Sidoarjo Sub-districtRegency, East Java, Indonesia.

MATERIALS AND METHODS

Ethical approval

The Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga (number 678-KE) reviewed and approved this study.

Parasite collection

The sample used comes from the Oriental rat snake Snake (*P. mucosa*), which is often caught and sold for its meat and skin through collectors. The collection was conducted at a snake slaughterhouse in Tulangan Sub-districtDistrict, Sidoarjo DistrictRegency, East Java Province, Indonesia. The presence of pentastomid parasites was observed in the respiratory tract of 60 slaughtered snakes. Pentastomides were taken and cleaned with distilled water, then stored in sterile plastic pots. The lungs of the snakes infected with the parasite were also isolated for histopathological preparation.

Prevalence calculation

The following formula calculated the prevalence of pentastomiasis in snake samples:

$$\text{Prevalence (\%)} = \frac{\text{(The number of snakes infected with pentastomids)}}{\text{(Total sample of snakes)}} \times 100\%.$$

Semichen-acetic carmine staining

The collected pentastomids were stained with the Semichen-Acetic Carmine technique (Kuhlmann 2006). Adult pentastomids were fixed on object glasses and soaked in 5% glycerin alcohol for 24 hours. Then they were soaked in 70% alcohol for five minutes. Subsequently, the object glasses with pentastomids were placed in Carmine alcohol solution (1:2) for eight hours. Next, the pentastomids were soaked in acid alcohol for 10 minutes and moved to base alcohol for 20 minutes. Furthermore, graded dehydration was carried out with 70%, 85%, and 95% alcohol for five minutes. The mounting step was done using Hung's I for 20 minutes. Finally, pentastomids were taken and placed on new clean object glasses. The pentastomids were dripped with Hung's II solutions and then covered with a cover glass.

Parasite identification

Characteristics of the head and body shape and body size of the parasite were identified based on Riley and Self (1980), Almedia et al. (2006), and Christoffersen and De Assis (2013; 2015). Identification of parasite egg size was carried out according to Keegan (1943).

The fresh parasite's length was measured from the anterior edge to the posterior end. The head was observed under a stereo microscope and documented with a Canon Digital Single-Lens Reflex (DSLR) camera. Furthermore, stained parasites were observed using a light microscope with 40-100× magnification and documented using Miconos Optilab. Some female parasites were incubated in PBS overnight at 37°C to collect the eggs. The supernatants were centrifuged at 1,500 rpm for 10 minutes, and the pellet was dripped onto a glass object and observed using a light microscope with 100× magnification. Those eggs were then measured using Miconos Optilab.

Histopathological preparations

The lungs of snakes with pentastomid infestation were fixed in 10% formalin. Then graded dehydration was carried out with serial alcohol concentrations (70%, 80%, 90%, 96%, and absolute) and clearing using xylol. Furthermore, paraffin embedding was performed to obtain a solid block of tissue. The tissue blocks were sliced (3 micrometers thick) using a microtome, mounted on an object glass, and stained using Hematoxylin and Eosin. Microscopic examination was performed using Nikon Eclipse Ci.

RESULTS AND DISCUSSIONS

The prevalence of pentastomiasis in *P. mucosa* snakes

Thirty-nine (or 65%) of the respiratory tract of 60 samples were positively infected with adult pentastomids; a

total of 204 pentastomids samples were collected (Table 1). Pentastomida was found to be strongly attached to the trachea and lungs of snakes (Figure 1). Some pentastomids even buried their heads in the lungs of snakes. The parasites of all snakes positively infected with pentastomids were found in the lungs (39 snakes), while only five (5) snakes which parasites spread to their tracheas.

This is the first study to determine the prevalence of pentastomiasis in *P. mucosa* in Sidoarjo, East Java, Indonesia. Our results showed that pentastomids were present in 39 of 60 (65%) snakes (Table 1). This finding shows the prevalence of pentastomiasis in *P. mucosa* was quite high. This study's prevalence is similar to pentastomiasis in Brazil that 8 of 15 or 53.33% of snakes were positively infected with pentastomida. The food consumed by the snakes probably caused the high prevalence in this research. The type of food is very influential on the prevalence of infection and the type of pentastomida that infected the snakes (Almeida et al. 2007). *P. mucosa* snakes usually feed on rats, frogs, toads, lizards, and even smaller non-venomous and smaller snakes (Sidik 2006). In addition, according to Esslinger (1962), rats, frogs, toads, lizards, and even non-venomous snakes can act as the intermediate hosts of pentastomida.

Identification of pentastomida

The characteristics of pentastomid found in this study can be seen in Table 2 and Figure 2. Pentastomids have a long cylindrical body with a larger head, a flat shape on the ventral, and a tapered tail or posterior. Their body was soft and whitish with a colorless cuticle, which tended to be transparent to make the internal organs visible. There were annuli throughout their body up to the posterior. There are two pairs of hooks (a total of four) around the head that attach to the host's lung tissue, and it has an ovoid-shaped mouth between the two pairs of hooks.

Both sexes, adult males and female pentastomids were found in this research (Figures 1 and 2). The number of male pentastomid was less (21 heads) than that of females (183 heads) Table 2. Table 2 also describes the size of each pentastomid. The female pentastomids had longer and larger bodies than the males (Figures 1 and 2). The length of the female bodies was 72-125 mm, with an average of 94.5 mm. Their abdominal diameter was 3.5-5 mm, averaging 4.2 mm. Furthermore, the anterior section or cephalothorax of the females was globular, with a neck separating their head and abdomen (Figure 3A). The average length and width of the female mouths were 0.24 mm and 0.16 mm. Furthermore, the results of the microscopy examination using Carmine staining showed that the female pentastomid had two spermathecae on the anterior part. That connects directly to the uterus that contains the egg (Figures 3C, 4B, and 4C). In addition, the vagina was in the posterior body and around the anus (Figure 4B).

On the other hand, the length of the males' bodies was 17-28.5 mm, with an average of 23.75 mm. Their abdominal diameter was 1.8-2.2 mm, averaging 2 mm. The average length and width of the mouth were 0.23 and 0.14

mm, respectively. The male body was simple and straight without any curves in the neck (Figure 3B). The genital pore was identified in the first annulus, and there was a pair of seminal vesicles, vas deferens, and cirrus (4D).

The eggs were oval and consisted of two layers of membrane that clearly could be distinguished. The first layer (outer membrane) was thin, while the second layer (inner membrane) was thick and yellowish. There was fluid-filled space between the first and second layers. The length of the eggs was 114-130 μm with an average of 118 μm , and the width was 108-124 μm with an average of 116 μm (Figure 3D).

Table 1. Prevalence of pentastomiasis in *P. mucosa* based on site and the number of parasites.

Sites	Positive (%)	Number of parasites
Lungs only	57% (34 of 60)	2-9 with an average of 5 Pentastomida
Trachea only	0% (0 of 60)	-
Lungs and Trachea	8% (5 of 60)	7-9 with an average of 8 Pentastomida
Total	65% (39 of 60)	204 pentastomida



Figure 1. Pentastomid infestation in the lungs of *P. mucosa*. The white arrows show areas with raised lesions around the attachment of pentastomids to the tissue. The black arrows show female pentastomids, and the red arrow shows male pentastomids.

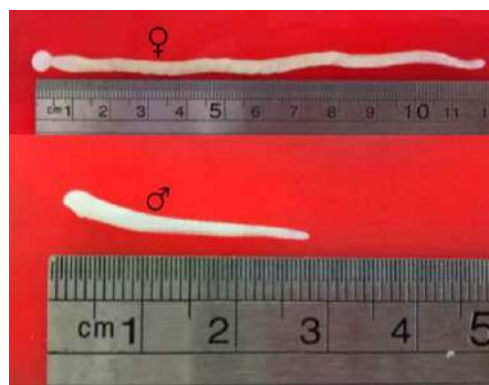


Figure 2. Differences in size between females (top) and males (below).

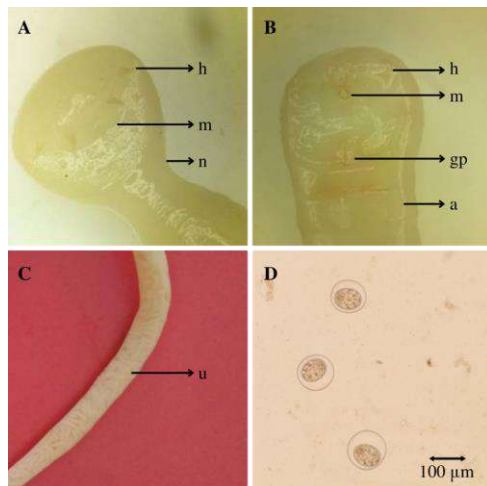


Figure 3. Pentastomida without staining. (A) The anterior section of the female; (B) The anterior section of the male; (C) Female body; (D) Pentastomid's eggs. h: hook, m: mouth, n: neck, gp: genital pore, a: first annuli, u: uterus.

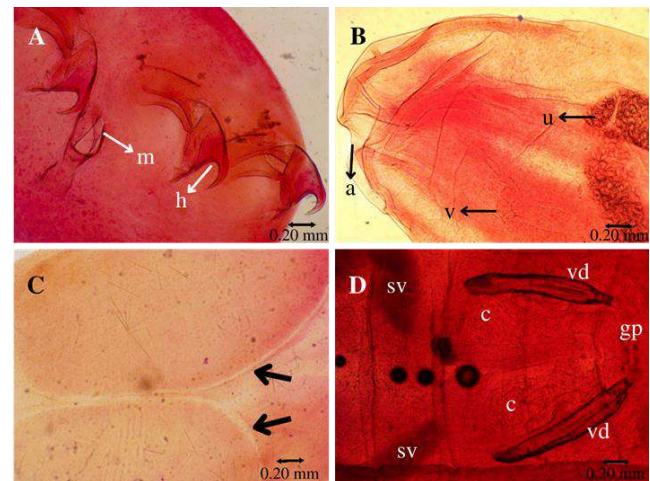


Figure 4. Carmine-stained pentastomida. (A) The anterior section; (B) The posterior section of the female; (C) A pair of female spermatheca; (D) Male reproductive organs. m: mouth, h: hook, a: anus, v: vagina, u: uterus, sv: seminal vesicle, c: cirrus, vd: vas deferens.

Table 2. The characteristics of pentastomida compared with *Kirichephalus pattoni* Stephens 1908 by Riley and Self (1980) and Keegan (1943).

Parameters	The current study		British Museum collection (Riley and Self 1980)		Keegan 1943
	Females	Males	Females	Males	
Total number of parasites	183	21	17	2	-
Body length	72-125 mm (\bar{X} 94.5 mm)	17-28.5 mm (\bar{X} 23.75 mm)	83-137 mm (\bar{X} 100 mm)	28-29 mm (\bar{X} 28.5 mm)	-
Number of <i>annuli</i>	29-41 (\bar{X} 31.6)	28-35 (\bar{X} 31)	34-38 (\bar{X} 36.5)	31-32 (\bar{X} 31.5)	-
Diameter of cephalothorax	4-5.5 mm (\bar{X} 4.75 mm)	2-2.5 mm (\bar{X} 2 mm)	4 mm	-	-
Neck	2-3 mm (\bar{X} 2.5 mm)	-	2 mm	-	-
Diameter of abdomen	3.5-5 mm (\bar{X} 4.2 mm)	1.8-2.2 mm (\bar{X} 2 mm)	4 mm	-	-
Hook parameter:					
AC					
CB	0.23-0.55 mm	0.22-0.35 mm			
AD	0.37-0.69 mm	0.29-0.35 mm			
	0.47-0.88 mm	0.29-0.45 mm			
Mouth:					
Length	0.16-29 mm	0.14-0.38 mm	-	-	-
Width	0.12-0.24 mm	0.12-0.18 mm			
Egg:					
Length	114-130 µm (\bar{X} 118 µm)	-	-	-	125-154 µm (\bar{X} 133 µm)
Width	108-124 µm (\bar{X} 116 µm)				106-143 µm (\bar{X} 123 µm)

Pentastomida was mostly found in the lungs, and the parasites also spread to the trachea in five snakes. This finding was similar to research by Riley and Self (1980) that revealed that adult *K. pattoni* (one of the pentastomida species) were only found in the lungs of *P. mucosa* snakes. In our observation, pentastomids that spread to the trachea were females. According to Sundar et al. (2015), the adult

pentastomids with mature eggs are expelled from the trachea and eliminated from the definitive host by oral expulsion or may also be swallowed. That results in shed eggs in the feces, which another intermediate mammal host will ingest to continue their life cycles.

The number of male pentastomids was fewer than the number of female ones. This case was also experienced by

John and Nadakal (1988) and Riley and Self (1980), who only found a few males compared the female. This study result follows Almeida et al. (2006), whose number of pentastomids and their species is unknown in biology

The taxonomy of pentastomida is still controversial. Moreover, it has been known through molecular data that pentastomida are closely related to crustaceans and belong to the class Maxillopoda, sub-phylum Crustacea, and the phylum Arthropoda (Paré 2008). Based on the egg size (Almeida et al. 2007); the female head form that globular with a neck separating the head from its body (Paré 2008); and the size of the body length, the number of the annuli, the diameter of the cephalothorax, neck, abdominal diameter, head, and body shape (Riley and Self 1979; Riley and Self 1980); pentastomida was classified as *K. pattoni*. *K. pattoni* can be classified as Porochepalida (Paré 2008). In addition, according to Christoffersen and De Assis (2013; 2015), pentastomid in *P. mucosa* snakes are classified into *K. pattoni*.

K. pattoni has a long annulated body, a tapered tail, an anal canal, two pairs of hooks or four hooks on its head, and a small ovoid-shaped mouth (Riley and Self 1979). The female *K. pattoni* has a larger body than the male one (Paré 2008). Female *K. pattoni* also has a spiral body with a larger head than its body, and a curve of the neck, whereas male one has a straight body without a curve on the neck (Riley and Self 1979; Riley and Self 1980; John and Nadakal 1988; Almeida et al. 2007). The spiral body in females is likely to facilitate their movement into the lung tissue of the definitive hosts (John and Nadakal 1988).

The egg morphology is similar to the eggs of *P. crotali* described by Sundar et al. (2015). These eggs were composed of two-layer membranes, of which the outer one was thin and the inner membrane thick, and there was a fluid-filled space between those two membranes. But the average diameter of the eggs measured in this investigation is smaller (118x116 µm) than the eggs of *P. crotali* (144 µm), as described by Sundar et al. (2015).

Histopathological changes in the lungs of *P. mucosa*

Squamates typically have two lungs; however, the left lung in most snakes is greatly reduced, never exceeding 85% of the right lung's size or absence (Funk and Bogan 2019). The right lung usually runs from near the heart to the cranial to the right kidney (Funk and Bogan 2019). The cranial portion of a lung is vascularized and functions primarily as a gas exchanger (vascular lung), whereas the caudal primarily functions as an air sac (saccular lung). The vascular lung's wall comprises honeycombed units called faveoli, allowing gas exchange (Funk and Bogan 2019). The faveoli in the snake lung parenchyma are lined microscopically by squamous epithelial cells (type I cells). In addition to a lesser degree, by cuboidal epithelial cells (type II) (Jacobson et al. 2021).

In this study, a nymphal stage of the parasite was found in the pulmonary tissue infected with pentastomida. The parasite is surrounded by some connective tissue (Figure 5). Finding the stages of the nymph in the snake's histological lung and the parasite's stages in the macroscopic lung organ means that the *P. mucosa* snake

may act as an intermediate and final host of *K. pattoni*. Within the lung cavities of the definitive host, the nymphs develop into adults (Sundar et al. 2015). In this study, pentastomida infection in the lungs only caused a mild histological reaction in the form of fibrosis in the area around the parasite.

Pentastomida has an indirect life cycle that typically utilizes vertebrate hosts. Consequently, these species rely on reptiles as their definitive host. The adults are usually found in their lungs (Wellehan and Walden 2019). Pentastomida parasites migrate extensively through the visceral before settling in the lungs and, less typically, the trachea and nasal passageways. Pentastomida can produce localized lesions, while molting can elicit antigenic reactions. Adults feed on lung tissue fluids and pass embryonated eggs, which are coughed up through the oral cavity, swallowed, and passed in the feces (Comolli and Divers 2021). In squamates, the pulmonary lumen is large, and the tracheal diameter may be smaller than that of the pentastomids. That poses a risk of respiratory obstruction by the adult pentastomids. However, the greatest pathological changes are seen in the intermediate host, where they may become embedded in tissues (Wellehan and Walden 2019).

Pentastomiasis in reptiles, especially snakes, occurs subclinically. Although the infection is large, death is usually associated with secondary septicemia and pneumonia (Tappe and Büttner 2009). Because pentastomids attach to the lung tissue, they can cause perforation, hemorrhaging, and serious inflammation in the host (Fernando and Fernando 2014). In a Common Garter Snake (*Thamnophis sirtalis* Linnaeus 1758), lung lesions were associated with one of the pentastomida species, *Kiricephalus coarctatus* Diesing 1850, where the embedded parasite had gotten into the hypaxial muscle. Infections with *Armillifer armillatus* Wyman 1845 have also been linked to parasitic pneumonia in gaboon vipers. Pentastomids occluding the trachea have been implicated in snake deaths. Bacterial infections could develop due to lung damage. For example, there are four out of nine *Boa constrictors* with pulmonary damage from *Porocephalus dominicana* Riley & Walters 1980 infection perished due to bacteremia and pericarditis (Walden et al. 2021).

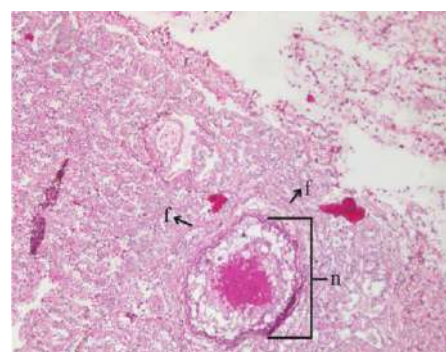


Figure 5. Histological microscopic picture of nymph-infected pulmo from pentastomida showed the presence of fibroblast around the area of infection. f: fibroblast, n: the body of the nymph stage pentastomida.

Bigger implications regarding possible impacts on human health may be anticipated based on the histopathological changes found in this study. Humans are the final hosts of pentastomids, which can be contracted from improperly cooked meat from infected snakes or drinking water contaminated with pentastomida eggs. In addition, humans can get the infection when handling and harvesting snakeskin or having direct contact with the mouth of a pet snake (Latif et al. 2016). The pathological consequences of the clinical symptoms experienced by infected humans may be the same or even more severe. That depends on the number of parasite eggs ingested, the duration from the larvae migrating to various organs to maturity and reproduction, and how severe the parasite infestation is in the human viscera.

Personal hygiene measures are required to prevent human infections. For example, avoid drinking river water directly or boiling the water before drinking. In addition, authorities should provide health education to warn people about eating undercooked snake meat, the possibility of transmission after handling snakes, and avoiding contact with snake excretions.

In conclusion the prevalence of pentastomiasis in slaughtered *P. mucosa* in Sidoarjo was 65% (39 of 60 snakes). The pentastomids that infect these snakes are classified as *K. pattoni*. The pulmonary histological findings only show a light reaction in the form of mild fibrosis.

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First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia

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Abstract. Plumeriastuti H, Pranashinta GT, Suwanti LT, Proboningrat A, Kusnoto. 2023. First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia. *Biodiversitas* 24: 2045-2051. Currently, reptiles, especially snakes, are increasingly popular as pets worldwide and are often consumed by several communities in Indonesia. This trend needs attention from a public health perspective because it can potentially cause zoonotic diseases, including parasitic disease such as pentastomiasis. This study aimed to determine: the prevalence of pentastomiasis, identify the parasite and observe the pulmonary histopathology in Oriental Rat Snakes (*Ptyas mucosa* Linnaeus 1758) collected in Tulangan, Sidoarjo, Indonesia. The methods used were calculating the prevalence of pentastomiasis, semichen-acetic carmine staining, and identifying parasites and eggs based on references as a comparison. Furthermore, histopathological preparation and hematoxylin-eosin staining were performed on the lungs infected with the parasite to observe the microscopic lesions. The results showed that 65% of the snakes had pentastomiasis, and the parasites were called *Kiricephalus pattoni* Stephens 1908. Furthermore, a microscopical examination revealed mild fibrosis in the pulmonary area due to pentastomid infestation. This study is the first reported pentastomida in *P. mucosa* in Sidoarjo, Indonesia, showing that snakes caught and commonly consumed were infected with zoonotic pentastomic.

Keywords: Colubrid snake, parasitic pneumonia, tongue worms, zoonotic parasitoses

INTRODUCTION

Exotic animals such as reptiles, especially various snakes, are now popular as pets worldwide (Okulewicz et al. 2014). However, they are also concerned with the increasing animal welfare and health issues through the animal trade in the market (Yudhana et al. 2021). Some species of snake are bred in captivity, while others are caught in the wild and sold as pets or bushmeat for consumption (Hardi et al. 2017; Yudhana et al. 2021).

Ptyas mucosa Linnaeus 1758 is a well-known snake species in Indonesia (Pranashinta et al. 2017). This type of snake, known as the Oriental Rat Snake and Indian Rat Snake, belongs to the Colubridae family which is widely distributed throughout East, South, and Southeast Asia (Auliya 2010; Muliya and Bhat 2016; Yuan et al. 2022). *P. mucosa* is a diurnal and semi-arboreal snake species that live on forest floors, agricultural fields, wet ground, and near human habitats. It feeds on frogs, rats, and other small animals (Chittora et al. 2021).

The meat of *P. mucosa* is used in the illegal trade to China (Auliya 2010). The meat and internal organs are used as fresh or frozen food, the bile and blood are used in traditional medicine, and the skin is exported to several European countries as raw materials for the leather industry (Pranashinta et al. 2017). Although illegal, most rural

communities in Indonesia trade and consume the meat and bile of *P. mucosa* for some reasons (Auliya 2010; Pranashinta et al. 2017).

Wildlife meat, however, poses a threat of transmitting infection with novel and exotic pathogens to human hosts (Hardi et al. 2017). Then new zoonotic diseases are likely to emerge consequently. For instance, Bats (Chiroptera) are important reservoirs of several emerging diseases, including Ebola Virus Disease (EVD), Severe Acute Respiratory Syndrome (SARS), and Middle East Respiratory Syndrome (MERS), as well as the current pandemic COVID-19 caused by SARS Coronavirus 2 (Irving et al. 2021). Moreover, many parasites also cause emerging zoonotic diseases, resulting in a significant public health burden, particularly in tropical countries (Alasil and Abdullah 2019).

Several parasites in confined reptiles spend a portion of their life cycle in the respiratory system. Most respiratory involvement is asymptomatic and mild, generating mostly localized inflammation except for excessive parasite loads. However, severe disease can result from high burdens or unusual host-parasite interactions, frequently worsened by secondary infections (Comolli 2021). Reptile-borne pentastomiasis is an unusual tropical disease neglected and under-researched in Asia, Africa, and South America (Sulyok et al. 2014).

Pentastomiasis is a parasitic zoonosis caused by infection with pentastomic, belonging to the subclass Pentastomida, under the phylum Arthropoda (Tongtako et al. 2013; Chittora et al. 2021; WHA 2019). Pentastomiasis is also known as tongue worms since the adult phase of the genus *Linguatula* has similarities with mammalian tongues (Paré 2008). Adult parasites are usually found in the respiratory tract of reptiles, birds, and mammals, including humans, while their larval forms are in the internal organs of insects, mammals, and amphibians (Paré 2008; Chen et al. 2010; Kelehear et al. 2014). Some zoonotic Pentastomida in snakes is *Armillifer* and *Porocephalus crotali* Humboldt 1812 (Rataj et al. 2011). Humans can be infected with pentastomic after they consume raw snake meat, have direct contact with the snake, drink water contaminated with parasitic eggs, or are contaminated through the feces (Kelehear et al. 2014; Tappe et al. 2016; Mendoza-Roldan et al. 2020).

Pentastomiasis in snakes have been reported in many countries, such as poisonous Terciopelo (*Bothrops asper* Garman 1883) in Costa Rica (Alvarado et al. 2015), Royal Python Snakes (*Python regius* Shaw 1802) in Nigeria (Ayinmode et al. 2010), Rat Snakes (*P. mucosa*) in Sri Lanka and India (Fernando and Fernando 2014; Sundar et al. 2015), wild snakes in Australia (Kelehear et al. 2014), various species of snakes in Slovenia and Pakistan (Rataj et al. 2011), Chinese Moccasin (*Agkistrodon acutus*) and Indian Python (*Python molurus* Linnaeus 1758) in China (Chen et al. 2010), and Totonacan Rattlesnake (*Crotalus totonacus* Gloyd & Kauffeld 1940) in Mexico (Tepos-Ramírez et al. 2022).

Moreover, to our best knowledge, snakes in Indonesia have no reports of pentastomic infections. Therefore, this study reported the prevalence and identified pentastomida and the pulmonary histopathological profiles in Oriental Rat Snakes (*P. mucosa*) in Sidoarjo Regency, East Java, Indonesia.

MATERIALS AND METHODS

Ethical approval

The Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga (number 678-KE) reviewed and approved this study.

Parasite collection

The sample used comes from the Oriental Rat Snake (*P. mucosa*), which is often caught and sold for its meat and skin through collectors. The collection was conducted at a snake slaughterhouse in Tulangan District, Sidoarjo Regency, East Java Province, Indonesia. The presence of pentastomid parasites was observed in the respiratory tract of 60 slaughtered snakes. Pentastomides were taken and cleaned with distilled water, then stored in sterile plastic pots. The lungs of the snakes infected with the parasite were also isolated for histopathological preparation.

Prevalence calculation

The following formula calculated the prevalence of pentastomiasis in snake samples:

Prevalence (%) = (The number of snakes infected with pentastomids) / (Total sample of snakes) × 100%.

Semichen-acetic carmine staining

The collected pentastomids were stained with the Semichen-Acetic Carmine technique (Kuhlmann 2006). Adult pentastomids were fixed on object glasses and soaked in 5% glycerin alcohol for 24 hours. Then they were soaked in 70% alcohol for five minutes. Subsequently, the object glasses with pentastomids were placed in Carmine alcohol solution (1:2) for eight hours. Next, the pentastomids were soaked in acid alcohol for 10 minutes and moved to base alcohol for 20 minutes. Furthermore, graded dehydration was carried out with 70%, 85%, and 95% alcohol for five minutes. The mounting step was done using Hung's I for 20 minutes. Finally, pentastomids were taken and placed on new clean object glasses. The pentastomids were dripped with Hung's II solutions and then covered with a cover glass.

Parasite identification

Characteristics of the head and body shape and body size of the parasite were identified based on Riley and Self (1980), Almedia et al. (2006), and Christoffersen and De Assis (2013; 2015). Identification of parasite egg size was carried out according to Keegan (1943).

The fresh parasite's length was measured from the anterior edge to the posterior end. The head was observed under a stereo microscope and documented with a Canon Digital Single-Lens Reflex (DSLR) camera. Furthermore, stained parasites were observed using a light microscope with 40-100× magnification and documented using Miconos Optilab. Some female parasites were incubated in PBS overnight at 37°C to collect the eggs. The supernatants were centrifuged at 1,500 rpm for 10 minutes, and the pellet was dripped onto a glass object and observed using a light microscope with 100× magnification. Those eggs were then measured using Miconos Optilab.

Histopathological preparations

The lungs of snakes with pentastomid infestation were fixed in 10% formalin. Then graded dehydration was carried out with serial alcohol concentrations (70%, 80%, 90%, 96%, and absolute) and clearing using xylol. Furthermore, paraffin embedding was performed to obtain a solid block of tissue. The tissue blocks were sliced (3 micrometers thick) using a microtome, mounted on an object glass, and stained using Hematoxylin and Eosin. Microscopic examination was performed using Nikon Eclipse Ci.

RESULTS AND DISCUSSIONS

The prevalence of pentastomiasis in *P. mucosa* snakes

Thirty-nine (or 65%) of the respiratory tract of 60 samples were positively infected with adult pentastomids; a

total of 204 pentastomids samples were collected (Table 1). Pentastomida was found to be strongly attached to the trachea and lungs of snakes (Figure 1). Some pentastomids even buried their heads in the lungs of snakes. The parasites of all snakes positively infected with pentastomids were found in the lungs (39 snakes), while only five (5) snakes which parasites spread to their tracheas.

This is the first study to determine the prevalence of pentastomiasis in *P. mucosa* in Sidoarjo, East Java, Indonesia. Our results showed that pentastomids were present in 39 of 60 (65%) snakes (Table 1). This finding shows the prevalence of pentastomiasis in *P. mucosa* was quite high. This study's prevalence is similar to pentastomiasis in Brazil that 8 of 15 or 53.33% of snakes were positively infected with pentastomida. The food consumed by the snakes probably caused the high prevalence in this research. The type of food is very influential on the prevalence of infection and the type of pentastomida that infected the snakes (Almeida et al. 2007). *P. mucosa* snakes usually feed on rats, frogs, toads, lizards, and even smaller non-venomous and smaller snakes (Sidik 2006). In addition, according to Esslinger (1962), rats, frogs, toads, lizards, and even non-venomous snakes can act as the intermediate hosts of pentastomida.

Identification of pentastomida

The characteristics of pentastomid found in this study can be seen in Table 2 and Figure 2. Pentastomids have a long cylindrical body with a larger head, a flat shape on the ventral, and a tapered tail or posterior. Their body was soft and whitish with a colorless cuticle, which tended to be transparent to make the internal organs visible. There were annuli throughout their body up to the posterior. There are two pairs of hooks (a total of four) around the head that attach to the host's lung tissue, and it has an ovoid-shaped mouth between the two pairs of hooks.

Both sexes, adult males and female pentastomids were found in this research (Figures 1 and 2). The number of male pentastomid was less (21 heads) than that of females (183 heads) Table 2. Table 2 also describes the size of each pentastomid. The female pentastomids had longer and larger bodies than the males (Figures 1 and 2). The length of the female bodies was 72-125 mm, with an average of 94.5 mm. Their abdominal diameter was 3.5-5 mm, averaging 4.2 mm. Furthermore, the anterior section or cephalothorax of the females was globular, with a neck separating their head and abdomen (Figure 3A). The average length and width of the female mouths were 0.24 mm and 0.16 mm. Furthermore, the results of the microscopy examination using Carmine staining showed that the female pentastomid had two spermathecae on the anterior part. That connects directly to the uterus that contains the egg (Figures 3C, 4B, and 4C). In addition, the vagina was in the posterior body and around the anus (Figure 4B).

On the other hand, the length of the males' bodies was 17-28.5 mm, with an average of 23.75 mm. Their abdominal diameter was 1.8-2.2 mm, averaging 2 mm. The average length and width of the mouth were 0.23 and 0.14 mm, respectively. The male body was simple and straight without any curves in the neck (Figure 3B). The genital

pore was identified in the first annulus, and there was a pair of seminal vesicles, vas deferens, and cirrus (4D).

The eggs were oval and consisted of two layers of membrane that clearly could be distinguished. The first layer (outer membrane) was thin, while the second layer (inner membrane) was thick and yellowish. There was fluid-filled space between the first and second layers. The length of the eggs was 114-130 μm with an average of 118 μm , and the width was 108-124 μm with an average of 116 μm (Figure 3D).

Table 1. Prevalence of pentastomiasis in *P. mucosa* based on site and the number of parasites

Sites	Positive (%)	Number of parasites
Lungs only	57% (34 of 60)	2-9 with an average of 5 Pentastomida
Trachea only	0% (0 of 60)	-
Lungs and Trachea	8% (5 of 60)	7-9 with an average of 8 Pentastomida
Total	65% (39 of 60)	204 pentastomida



Figure 1. Pentastomid infestation in the lungs of *P. mucosa*. The white arrows show areas with raised lesions around the attachment of pentastomids to the tissue. The black arrows show female pentastomids, and the red arrow shows male pentastomids

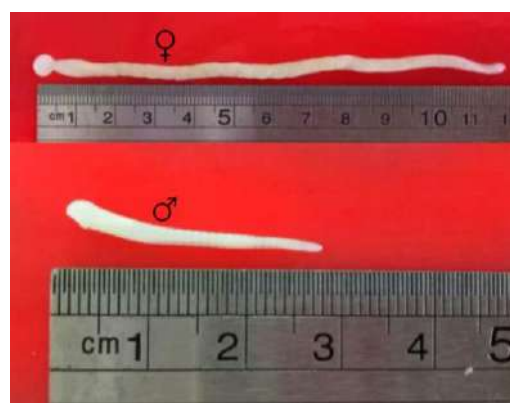


Figure 2. Differences in size between females (top) and males (below)

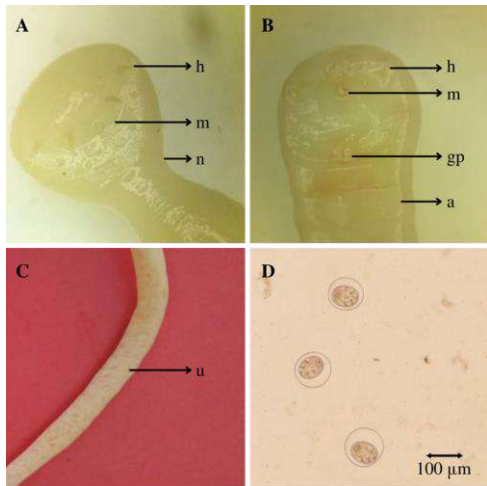


Figure 3. Pentastomida without staining. A. The anterior section of the female. B. The anterior section of the male. C. Female body. D. Pentastomid's eggs. h: hook, m: mouth, n: neck, gp: genital pore, a: first annuli, u: uterus

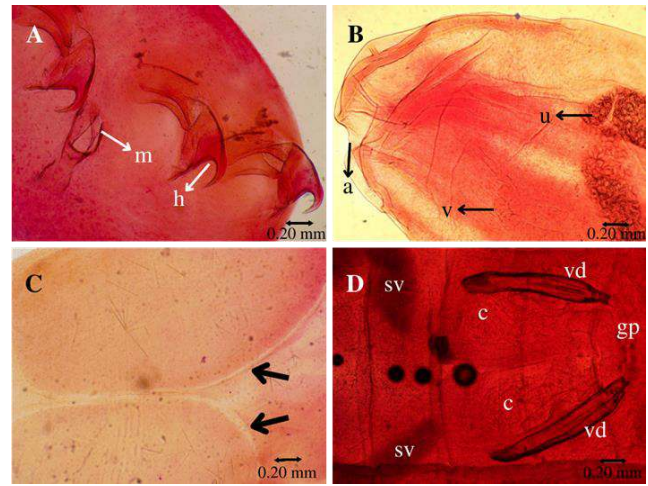


Figure 4. Carmine-stained pentastomida. A. The anterior section. B. The posterior section of the female. C. A pair of female spermatheca. D. Male reproductive organs. m: mouth, h: hook, a: anus, v: vagina, u: uterus, sv: seminal vesicle, c: cirrus, vd: vas deferens

Table 2. The characteristics of pentastomida compared with *Kirichephalus pattoni* Stephens 1908 by Riley and Self (1980) and Keegan (1943)

Parameters	The current study		British Museum collection (Riley and Self 1980)		Keegan 1943
	Females	Males	Females	Males	
Total number of parasites	183	21	17	2	-
Body length	72-125 mm (\bar{X} 94.5 mm)	17-28.5 mm (\bar{X} 23.75 mm)	83-137 mm (\bar{X} 100 mm)	28-29 mm (\bar{X} 28.5 mm)	-
Number of annuli	29-41 (\bar{X} 31.6)	28-35 (\bar{X} 31)	34-38 (\bar{X} 36.5)	31-32 (\bar{X} 31.5)	-
Diameter of cephalothorax	4-5.5 mm (\bar{X} 4.75 mm)	2-2.5 mm (\bar{X} 2 mm)	4 mm	-	-
Neck	2-3 mm (\bar{X} 2.5 mm)	-	2 mm	-	-
Diameter of abdomen	3.5-5 mm (\bar{X} 4.2 mm)	1.8-2.2 mm (\bar{X} 2 mm)	4 mm	-	-
Hook parameter:					
AC	0.23-0.55 mm	0.22-0.35 mm	-	-	-
CB	0.37-0.69 mm	0.29-0.35 mm	-	-	-
AD	0.47-0.88 mm	0.29-0.45 mm	-	-	-
Mouth:					
Length	0.16-29 mm	0.14-0.38 mm	-	-	-
Width	0.12-0.24 mm	0.12-0.18 mm	-	-	-
Egg:					
Length	114-130 µm (\bar{X} 118 µm)	-	-	-	125-154 µm (\bar{X} 133 µm)
Width	108-124 µm (\bar{X} 116 µm)	-	-	-	106-143 µm (\bar{X} 123 µm)

Pentastomida was mostly found in the lungs, and the parasites also spread to the trachea in five snakes. This finding was similar to research by Riley and Self (1980) that revealed that adult *K. pattoni* (one of the pentastomida species) were only found in the lungs of *P. mucosa* snakes. In our observation, pentastomids that spread to the trachea were females. According to Sundar et al. (2015), the adult pentastomids with mature eggs are expelled from the

trachea and eliminated from the definitive host by oral expulsion or may also be swallowed. That results in shed eggs in the feces, which another intermediate mammal host will ingest to continue their life cycles.

The number of male pentastomids was fewer than the number of female ones. This case was also experienced by John and Nadakal (1988) and Riley and Self (1980), who only found a few males compared the female. This study

result follows Almeida et al. (2006), whose number of pentastomids and their species is unknown in biology

The taxonomy of pentastomida is still controversial. Moreover, it has been known through molecular data that pentastomida are closely related to crustaceans and belong to the class Maxillopoda, sub-phylum Crustacea, and the phylum Arthropoda (Paré 2008). Based on the egg size (Almeida et al. 2007); the female head form that globular with a neck separating the head from its body (Paré 2008); and the size of the body length, the number of the annuli, the diameter of the cephalothorax, neck, abdominal diameter, head, and body shape (Riley and Self 1979; Riley and Self 1980); pentastomida was classified as *K. pattoni*. *K. pattoni* can be classified as Porocephalida (Paré 2008). In addition, according to Christoffersen and De Assis (2013; 2015), pentastomid in *P. mucosa* snakes are classified into *K. pattoni*.

K. pattoni has a long annulated body, a tapered tail, an anal canal, two pairs of hooks or four hooks on its head, and a small ovoid-shaped mouth (Riley and Self 1979). The female *K. pattoni* has a larger body than the male one (Paré 2008). Female *K. pattoni* also has a spiral body with a larger head than its body, and a curve of the neck, whereas male one has a straight body without a curve on the neck (Riley and Self 1979; Riley and Self 1980; John and Nadakal 1988; Almeida et al. 2007). The spiral body in females is likely to facilitate their movement into the lung tissue of the definitive hosts (John and Nadakal 1988).

The egg morphology is similar to the eggs of *P. crotali* described by Sundar et al. (2015). These eggs were composed of two-layer membranes, of which the outer one was thin and the inner membrane thick, and there was a fluid-filled space between those two membranes. But the average diameter of the eggs measured in this investigation is smaller (118x116 µm) than the eggs of *P. crotali* (144 µm), as described by Sundar et al. (2015).

Histopathological changes in the lungs of *P. mucosa*

Squamates typically have two lungs; however, the left lung in most snakes is greatly reduced, never exceeding 85% of the right lung's size or absence (Funk and Bogan 2019). The right lung usually runs from near the heart to the cranial to the right kidney (Funk and Bogan 2019). The cranial portion of a lung is vascularized and functions primarily as a gas exchanger (vascular lung), whereas the caudal primarily functions as an air sac (saccular lung). The vascular lung's wall comprises honeycombed units called faveoli, allowing gas exchange (Funk and Bogan 2019). The faveoli in the snake lung parenchyma are lined microscopically by squamous epithelial cells (type I cells). In addition to a lesser degree, by cuboidal epithelial cells (type II) (Jacobson et al. 2021).

In this study, a nymphal stage of the parasite was found in the pulmonary tissue infected with pentastomida. The parasite is surrounded by some connective tissue (Figure 5). Finding the stages of the nymph in the snake's histological lung and the parasite's stages in the macroscopic lung organ means that the *P. mucosa* snake may act as an intermediate and final host of *K. pattoni*. Within the lung cavities of the definitive host, the nymphs

develop into adults (Sundar et al. 2015). In this study, pentastomida infection in the lungs only caused a mild histological reaction in the form of fibrosis in the area around the parasite.

Pentastomida has an indirect life cycle that typically utilizes vertebrate hosts. Consequently, these species rely on reptiles as their definitive host. The adults are usually found in their lungs (Wellehan and Walden 2019). Pentastomida parasites migrate extensively through the visceral before settling in the lungs and, less typically, the trachea and nasal passageways. Pentastomida can produce localized lesions, while molting can elicit antigenic reactions. Adults feed on lung tissue fluids and pass embryonated eggs, which are coughed up through the oral cavity, swallowed, and passed in the feces (Comolli and Divers 2021). In squamates, the pulmonary lumen is large, and the tracheal diameter may be smaller than that of the pentastomids. That poses a risk of respiratory obstruction by the adult pentastomids. However, the greatest pathological changes are seen in the intermediate host, where they may become embedded in tissues (Wellehan and Walden 2019).

Pentastomiasis in reptiles, especially snakes, occurs subclinically. Although the infection is large, death is usually associated with secondary septicemia and pneumonia (Tappe and Büttner 2009). Because pentastomids attach to the lung tissue, they can cause perforation, hemorrhaging, and serious inflammation in the host (Fernando and Fernando 2014). In a Common Garter Snake (*Thamnophis sirtalis* Linnaeus 1758), lung lesions were associated with one of the pentastomida species, *Kiricephalus coarctatus* Diesing 1850, where the embedded parasite had gotten into the hypaxial muscle. Infections with *Armillifer armillatus* Wyman 1845 have also been linked to parasitic pneumonia in gaboon vipers. Pentastomids occluding the trachea have been implicated in snake deaths. Bacterial infections could develop due to lung damage. For example, there are four out of nine *Boa constrictors* with pulmonary damage from *Porocephalus dominicana* Riley & Walters 1980 infection perished due to bacteremia and pericarditis (Walden et al. 2021).

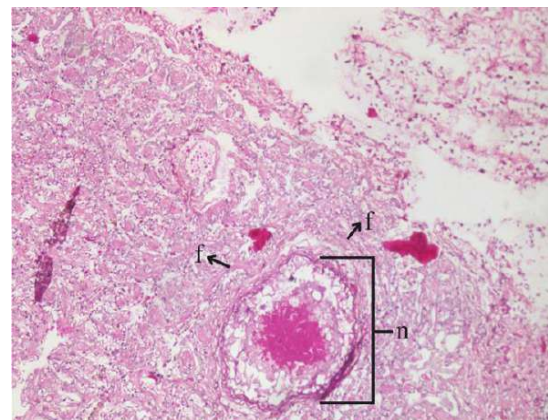


Figure 5. Histological microscopic picture of nymph-infected pulmo from pentastomida showed the presence of fibroblast around the area of infection. f: fibroblast, n: the body of the nymph stage pentastomida

Bigger implications regarding possible impacts on human health may be anticipated based on the histopathological changes found in this study. Humans are the final hosts of pentastomic, which can be contracted from improperly cooked meat from infected snakes or drinking water contaminated with pentastomida eggs. In addition, humans can get the infection when handling and harvesting snakeskin or having direct contact with the mouth of a pet snake (Latif et al. 2016). The pathological consequences of the clinical symptoms experienced by infected humans may be the same or even more severe. That depends on the number of parasite eggs ingested, the duration from the larvae migrating to various organs to maturity and reproduction, and how severe the parasite infestation is in the human viscera.

Personal hygiene measures are required to prevent human infections. For example, avoid drinking river water directly or boiling the water before drinking. In addition, authorities should provide health education to warn people about eating undercooked snake meat, the possibility of transmission after handling snakes, and avoiding contact with snake excretions.

In conclusion the prevalence of pentastomiasis in slaughtered *P. mucosa* in Sidoarjo was 65% (39 of 60 snakes). The pentastomids that infect these snakes are classified as *K. pattoni*. The pulmonary histological findings only show a light reaction in the form of mild fibrosis.

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First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia

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Abstract. Plumeriastuti H, Pranashinta GT, Suwanti LT, Proboningrat A, Kusnoto. 2023. First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia. *Biodiversitas* 24: 2045-2051. Currently, reptiles, especially snakes, are increasingly popular as pets worldwide and are often consumed by several communities in Indonesia. This trend needs attention from a public health perspective because it can potentially cause zoonotic diseases, including parasitic disease such as pentastomiasis. This study aimed to determine: the prevalence of pentastomiasis, identify the parasite and observe the pulmonary histopathology in Oriental Rat Snakes (*Ptyas mucosa* Linnaeus 1758) collected in Tulangan, Sidoarjo, Indonesia. The methods used were calculating the prevalence of pentastomiasis, semichen-acetic carmine staining, and identifying parasites and eggs based on references as a comparison. Furthermore, histopathological preparation and hematoxylin-eosin staining were performed on the lungs infected with the parasite to observe the microscopic lesions. The results showed that 65% of the snakes had pentastomiasis, and the parasites were called *Kiricephalus pattoni* Stephens 1908. Furthermore, a microscopical examination revealed mild fibrosis in the pulmonary area due to pentastomid infestation. This study is the first reported pentastomida in *P. mucosa* in Sidoarjo, Indonesia, showing that snakes caught and commonly consumed were infected with zoonotic pentastomic.

Keywords: Colubrid snake, parasitic pneumonia, tongue worms, zoonotic parasitoses

INTRODUCTION

Exotic animals such as reptiles, especially various snakes, are now popular as pets worldwide (Okulewicz et al. 2014). However, they are also concerned with the increasing animal welfare and health issues through the animal trade in the market (Yudhana et al. 2021). Some species of snake are bred in captivity, while others are caught in the wild and sold as pets or bushmeat for consumption (Hardi et al. 2017; Yudhana et al. 2021).

Ptyas mucosa Linnaeus 1758 is a well-known snake species in Indonesia (Pranashinta et al. 2017). This type of snake, known as the Oriental Rat Snake and Indian Rat Snake, belongs to the Colubridae family which is widely distributed throughout East, South, and Southeast Asia (Auliya 2010; Muliya and Bhat 2016; Yuan et al. 2022). *P. mucosa* is a diurnal and semi-arboreal snake species that live on forest floors, agricultural fields, wet ground, and near human habitats. It feeds on frogs, rats, and other small animals (Chittora et al. 2021).

The meat of *P. mucosa* is used in the illegal trade to China (Auliya 2010). The meat and internal organs are used as fresh or frozen food, the bile and blood are used in traditional medicine, and the skin is exported to several European countries as raw materials for the leather industry (Pranashinta et al. 2017). Although illegal, most rural

communities in Indonesia trade and consume the meat and bile of *P. mucosa* for some reasons (Auliya 2010; Pranashinta et al. 2017).

Wildlife meat, however, poses a threat of transmitting infection with novel and exotic pathogens to human hosts (Hardi et al. 2017). Then new zoonotic diseases are likely to emerge consequently. For instance, Bats (Chiroptera) are important reservoirs of several emerging diseases, including Ebola Virus Disease (EVD), Severe Acute Respiratory Syndrome (SARS), and Middle East Respiratory Syndrome (MERS), as well as the current pandemic COVID-19 caused by SARS Coronavirus 2 (Irving et al. 2021). Moreover, many parasites also cause emerging zoonotic diseases, resulting in a significant public health burden, particularly in tropical countries (Alasil and Abdullah 2019).

Several parasites in confined reptiles spend a portion of their life cycle in the respiratory system. Most respiratory involvement is asymptomatic and mild, generating mostly localized inflammation except for excessive parasite loads. However, severe disease can result from high burdens or unusual host-parasite interactions, frequently worsened by secondary infections (Comolli 2021). Reptile-borne pentastomiasis is an unusual tropical disease neglected and under-researched in Asia, Africa, and South America (Sulyok et al. 2014).

Pentastomiasis is a parasitic zoonosis caused by infection with pentastomic, belonging to the subclass Pentastomida, under the phylum Arthropoda (Tongtako et al. 2013; Chittora et al. 2021; WHA 2019). Pentastomiasis is also known as tongue worms since the adult phase of the genus *Linguatula* has similarities with mammalian tongues (Paré 2008). Adult parasites are usually found in the respiratory tract of reptiles, birds, and mammals, including humans, while their larval forms are in the internal organs of insects, mammals, and amphibians (Paré 2008; Chen et al. 2010; Kelehear et al. 2014). Some zoonotic Pentastomida in snakes is *Armillifer* and *Porocephalus crotali* Humboldt 1812 (Rataj et al. 2011). Humans can be infected with pentastomic after they consume raw snake meat, have direct contact with the snake, drink water contaminated with parasitic eggs, or are contaminated through the feces (Kelehear et al. 2014; Tappe et al. 2016; Mendoza-Roldan et al. 2020).

Pentastomiasis in snakes have been reported in many countries, such as poisonous Terciopelo (*Bothrops asper* Garman 1883) in Costa Rica (Alvarado et al. 2015), Royal Python Snakes (*Python regius* Shaw 1802) in Nigeria (Ayinmode et al. 2010), Rat Snakes (*P. mucosa*) in Sri Lanka and India (Fernando and Fernando 2014; Sundar et al. 2015), wild snakes in Australia (Kelehear et al. 2014), various species of snakes in Slovenia and Pakistan (Rataj et al. 2011), Chinese Moccasin (*Agkistrodon acutus*) and Indian Python (*Python molurus* Linnaeus 1758) in China (Chen et al. 2010), and Totonacan Rattlesnake (*Crotalus totonacus* Gloyd & Kauffeld 1940) in Mexico (Tepos-Ramírez et al. 2022).

Moreover, to our best knowledge, snakes in Indonesia have no reports of pentastomic infections. Therefore, this study reported the prevalence and identified pentastomida and the pulmonary histopathological profiles in Oriental Rat Snakes (*P. mucosa*) in Sidoarjo Regency, East Java, Indonesia.

MATERIALS AND METHODS

Ethical approval

The Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia (number 678-KE) reviewed and approved this study.

Parasite collection

The sample used comes from the Oriental Rat Snake (*P. mucosa*), which is often caught and sold for its meat and skin through collectors. The collection was conducted at a snake slaughterhouse in Tulangan District, Sidoarjo Regency, East Java Province, Indonesia. The presence of pentastomid parasites was observed in the respiratory tract of 60 slaughtered snakes. Pentastomides were taken and cleaned with distilled water, then stored in sterile plastic pots. The lungs of the snakes infected with the parasite were also isolated for histopathological preparation.

Prevalence calculation

The following formula calculated the prevalence of pentastomiasis in snake samples:

Prevalence (%) = (The number of snakes infected with pentastomids) / (Total sample of snakes) × 100%.

Semichen-acetic carmine staining

The collected pentastomids were stained with the Semichen-Acetic Carmine technique (Kuhlmann 2006). Adult pentastomids were fixed on object glasses and soaked in 5% glycerin alcohol for 24 hours. Then they were soaked in 70% alcohol for five minutes. Subsequently, the object glasses with pentastomids were placed in Carmine alcohol solution (1:2) for eight hours. Next, the pentastomids were soaked in acid alcohol for 10 minutes and moved to base alcohol for 20 minutes. Furthermore, graded dehydration was carried out with 70%, 85%, and 95% alcohol for five minutes. The mounting step was done using Hung's I for 20 minutes. Finally, pentastomids were taken and placed on new clean object glasses. The pentastomids were dripped with Hung's II solutions and then covered with a cover glass.

Parasite identification

Characteristics of the head and body shape and body size of the parasite were identified based on Riley and Self (1980), Almedia et al. (2006), and Christoffersen and De Assis (2013; 2015). Identification of parasite egg size was carried out according to Keegan (1943).

The fresh parasite's length was measured from the anterior edge to the posterior end. The head was observed under a stereo microscope and documented with a Canon Digital Single-Lens Reflex (DSLR) camera. Furthermore, stained parasites were observed using a light microscope with 40-100× magnification and documented using Miconos Optilab. Some female parasites were incubated in PBS overnight at 37°C to collect the eggs. The supernatants were centrifuged at 1,500 rpm for 10 minutes, and the pellet was dripped onto a glass object and observed using a light microscope with 100× magnification. Those eggs were then measured using Miconos Optilab.

Histopathological preparations

The lungs of snakes with pentastomid infestation were fixed in 10% formalin. Then graded dehydration was carried out with serial alcohol concentrations (70%, 80%, 90%, 96%, and absolute) and clearing using xylol. Furthermore, paraffin embedding was performed to obtain a solid block of tissue. The tissue blocks were sliced (3 micrometers thick) using a microtome, mounted on an object glass, and stained using Hematoxylin and Eosin. Microscopic examination was performed using Nikon Eclipse Ci.

RESULTS AND DISCUSSIONS

The prevalence of pentastomiasis in *P. mucosa* snakes

Thirty-nine (or 65%) of the respiratory tract of 60 samples were positively infected with adult pentastomids; a

total of 204 pentastomids samples were collected (Table 1). Pentastomida was found to be strongly attached to the trachea and lungs of snakes (Figure 1). Some pentastomids even buried their heads in the lungs of snakes. The parasites of all snakes positively infected with pentastomids were found in the lungs (39 snakes), while only five (5) snakes which parasites spread to their tracheas.

This is the first study to determine the prevalence of pentastomiasis in *P. mucosa* in Sidoarjo, East Java, Indonesia. Our results showed that pentastomids were present in 39 of 60 (65%) snakes (Table 1). This finding shows the prevalence of pentastomiasis in *P. mucosa* was quite high. This study's prevalence is similar to pentastomiasis in Brazil that 8 of 15 or 53.33% of snakes were positively infected with pentastomida. The food consumed by the snakes probably caused the high prevalence in this research. The type of food is very influential on the prevalence of infection and the type of pentastomida that infected the snakes (Almeida et al. 2007). *P. mucosa* snakes usually feed on rats, frogs, toads, lizards, and even smaller non-venomous and smaller snakes (Sidik 2006). In addition, according to Esslinger (1962), rats, frogs, toads, lizards, and even non-venomous snakes can act as the intermediate hosts of pentastomida.

Identification of pentastomida

The characteristics of pentastomid found in this study can be seen in Table 2 and Figure 2. Pentastomids have a long cylindrical body with a larger head, a flat shape on the ventral, and a tapered tail or posterior. Their body was soft and whitish with a colorless cuticle, which tended to be transparent to make the internal organs visible. There were annuli throughout their body up to the posterior. There are two pairs of hooks (a total of four) around the head that attach to the host's lung tissue, and it has an ovoid-shaped mouth between the two pairs of hooks.

Both sexes, adult males and female pentastomids were found in this research (Figures 1 and 2). The number of male pentastomid was less (21 heads) than that of females (183 heads) Table 2. Table 2 also describes the size of each pentastomid. The female pentastomids had longer and larger bodies than the males (Figures 1 and 2). The length of the female bodies was 72-125 mm, with an average of 94.5 mm. Their abdominal diameter was 3.5-5 mm, averaging 4.2 mm. Furthermore, the anterior section or cephalothorax of the females was globular, with a neck separating their head and abdomen (Figure 3.A). The average length and width of the female mouths were 0.24 mm and 0.16 mm. Furthermore, the results of the microscopy examination using Carmine staining showed that the female pentastomid had two spermathecae on the anterior part. That connects directly to the uterus that contains the egg (Figures 3.C, 4.B, and 4.C). In addition, the vagina was in the posterior body and around the anus (Figure 4.B).

On the other hand, the length of the males' bodies was 17-28.5 mm, with an average of 23.75 mm. Their abdominal diameter was 1.8-2.2 mm, averaging 2 mm. The average length and width of the mouth were 0.23 and 0.14 mm, respectively. The male body was simple and straight without any curves in the neck (Figure 3.B). The genital

pore was identified in the first annulus, and there was a pair of seminal vesicles, vas deferens, and cirrus (Figure 4.D).

The eggs were oval and consisted of two layers of membrane that clearly could be distinguished. The first layer (outer membrane) was thin, while the second layer (inner membrane) was thick and yellowish. There was fluid-filled space between the first and second layers. The length of the eggs was 114-130 μm with an average of 118 μm , and the width was 108-124 μm with an average of 116 μm (Figure 3.D).

Table 1. Prevalence of pentastomiasis in *P. mucosa* based on site and the number of parasites

Sites	Positive (%)	Number of parasites
Lungs only	57% (34 of 60)	2-9 with an average of 5 Pentastomida
Trachea only	0% (0 of 60)	-
Lungs and Trachea	8% (5 of 60)	7-9 with an average of 8 Pentastomida
Total	65% (39 of 60)	204 pentastomida



Figure 1. Pentastomid infestation in the lungs of *P. mucosa*. The white arrows show areas with raised lesions around the attachment of pentastomids to the tissue. The black arrows show female pentastomids, and the red arrow shows male pentastomids.

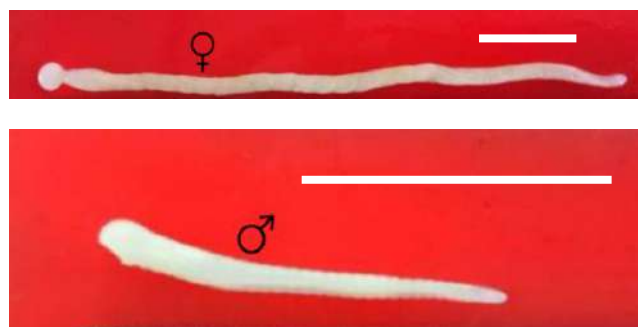


Figure 2. Differences in size between females (top) and males (below). Bar = 2 cm

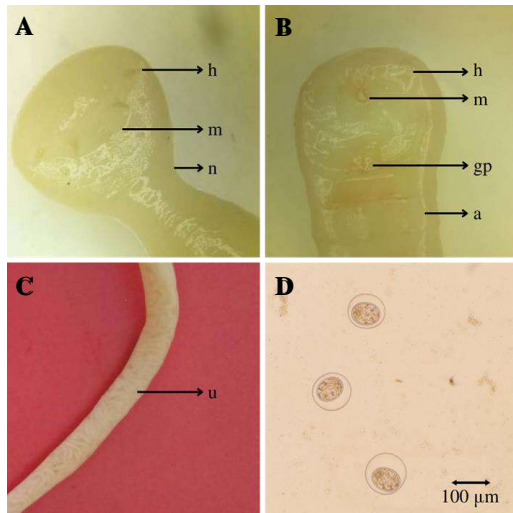


Figure 3. Pentastomida without staining. A. The anterior section of the female. B. The anterior section of the male. C. Female body. D. Pentastomid's eggs. h: hook, m: mouth, n: neck, gp: genital pore, a: first annuli, u: uterus

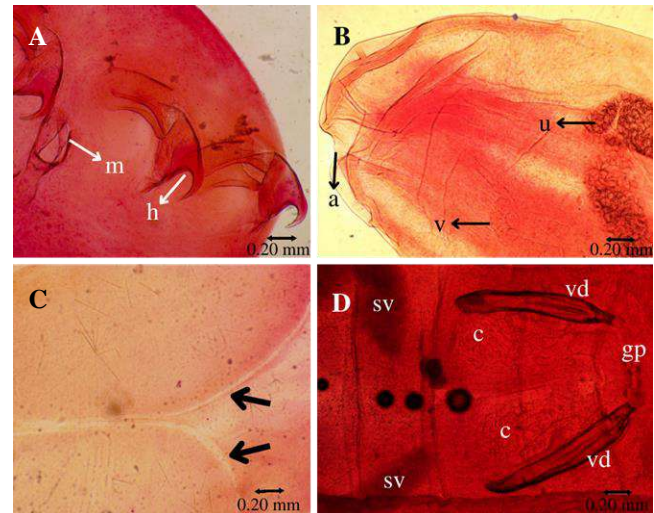


Figure 4. Carmine-stained pentastomida. A. The anterior section. B. The posterior section of the female. C. A pair of female spermatheca. D. Male reproductive organs. m: mouth, h: hook, a: anus, v: vagina, u: uterus, sv: seminal vesicle, c: cirrus, vd: vas deferens

Table 2. The characteristics of pentastomida compared with *Kirichephalus pattoni* Stephens 1908 by Riley and Self (1980) and Keegan (1943)

Parameters	The current study		British Museum collection (Riley and Self 1980)		Keegan 1943
	Females	Males	Females	Males	
Total number of parasites	183	21	17	2	-
Body length	72-125 mm (\bar{X} 94.5 mm)	17-28.5 mm (\bar{X} 23.75 mm)	83-137 mm (\bar{X} 100 mm)	28-29 mm (\bar{X} 28.5 mm)	-
Number of <i>annuli</i>	29-41 (\bar{X} 31.6)	28-35 (\bar{X} 31)	34-38 (\bar{X} 36.5)	31-32 (\bar{X} 31.5)	-
Diameter of cephalothorax	4-5.5 mm (\bar{X} 4.75 mm)	2-2.5 mm (\bar{X} 2 mm)	4 mm	-	-
Neck	2-3 mm (\bar{X} 2.5 mm)	-	2 mm	-	-
Diameter of abdomen	3.5-5 mm (\bar{X} 4.2 mm)	1.8-2.2 mm (\bar{X} 2 mm)	4 mm	-	-
Hook parameter:					
AC	0.23-0.55 mm	0.22-0.35 mm	-	-	-
CB	0.37-0.69 mm	0.29-0.35 mm	-	-	-
AD	0.47-0.88 mm	0.29-0.45 mm	-	-	-
Mouth:					
Length	0.16-29 mm	0.14-0.38 mm	-	-	-
Width	0.12-0.24 mm	0.12-0.18 mm	-	-	-
Egg:					
Length	114-130 µm (\bar{X} 118 µm)	-	-	-	125-154 µm (\bar{X} 133 µm)
Width	108-124 µm (\bar{X} 116 µm)	-	-	-	106-143 µm (\bar{X} 123 µm)

Pentastomida was mostly found in the lungs, and the parasites also spread to the trachea in five snakes. This finding was similar to research by Riley and Self (1980) that revealed that adult *K. pattoni* (one of the pentastomida species) were only found in the lungs of *P. mucosa* snakes. In our observation, pentastomids that spread to the trachea were females. According to Sundar et al. (2015), the adult pentastomids with mature eggs are expelled from the trachea and eliminated from the definitive host by oral

expulsion or may also be swallowed. That results in shed eggs in the feces, which another intermediate mammal host will ingest to continue their life cycles.

The number of male pentastomids was fewer than the number of female ones. This case was also experienced by John and Nadakal (1988) and Riley and Self (1980), who only found a few males compared the female. This study result follows Almeida et al. (2006), whose number of pentastomids and their species is unknown in biology.

The taxonomy of pentastomida is still controversial. Moreover, it has been known through molecular data that pentastomida are closely related to crustaceans and belong to the class Maxillopoda, sub-phylum Crustacea, and the phylum Arthropoda (Paré 2008). Based on the egg size (Almeida et al. 2007); the female head form that globular with a neck separating the head from its body (Paré 2008); and the size of the body length, the number of the annuli, the diameter of the cephalothorax, neck, abdominal diameter, head, and body shape (Riley and Self 1979; Riley and Self 1980); pentastomida was classified as *K. pattoni*. *K. pattoni* can be classified as Porocephalida (Paré 2008). In addition, according to Christoffersen and De Assis (2013; 2015), pentastomid in *P. mucosa* snakes are classified into *K. pattoni*.

K. pattoni has a long annulated body, a tapered tail, an anal canal, two pairs of hooks or four hooks on its head, and a small ovoid-shaped mouth (Riley and Self 1979). The female *K. pattoni* has a larger body than the male one (Paré 2008). Female *K. pattoni* also has a spiral body with a larger head than its body, and a curve of the neck, whereas male one has a straight body without a curve on the neck (Riley and Self 1979; Riley and Self 1980; John and Nadakal 1988; Almeida et al. 2007). The spiral body in females is likely to facilitate their movement into the lung tissue of the definitive hosts (John and Nadakal 1988).

The egg morphology is similar to the eggs of *P. crotali* described by Sundar et al. (2015). These eggs were composed of two-layer membranes, of which the outer one was thin and the inner membrane thick, and there was a fluid-filled space between those two membranes. But the average diameter of the eggs measured in this investigation is smaller (118x116 µm) than the eggs of *P. crotali* (144 µm), as described by Sundar et al. (2015).

Histopathological changes in the lungs of *P. mucosa*

Squamates typically have two lungs; however, the left lung in most snakes is greatly reduced, never exceeding 85% of the right lung's size or absence (Funk and Bogan 2019). The right lung usually runs from near the heart to the cranial to the right kidney (Funk and Bogan 2019). The cranial portion of a lung is vascularized and functions primarily as a gas exchanger (vascular lung), whereas the caudal primarily functions as an air sac (saccular lung). The vascular lung's wall comprises honeycombed units called faveoli, allowing gas exchange (Funk and Bogan 2019). The faveoli in the snake lung parenchyma are lined microscopically by squamous epithelial cells (type I cells). In addition to a lesser degree, by cuboidal epithelial cells (type II) (Jacobson et al. 2021).

In this study, a nymphal stage of the parasite was found in the pulmonary tissue infected with pentastomida. The parasite is surrounded by some connective tissue (Figure 5). Finding the stages of the nymph in the snake's histological lung and the parasite's stages in the macroscopic lung organ means that the *P. mucosa* snake may act as an intermediate and final host of *K. pattoni*. Within the lung cavities of the definitive host, the nymphs develop into adults (Sundar et al. 2015). In this study, pentastomida infection in the lungs only caused a mild

histological reaction in the form of fibrosis in the area around the parasite.

Pentastomida has an indirect life cycle that typically utilizes vertebrate hosts. Consequently, these species rely on reptiles as their definitive host. The adults are usually found in their lungs (Wellehan and Walden 2019). Pentastomida parasites migrate extensively through the visceral before settling in the lungs and, less typically, the trachea and nasal passageways. Pentastomida can produce localized lesions, while molting can elicit antigenic reactions. Adults feed on lung tissue fluids and pass embryonated eggs, which are coughed up through the oral cavity, swallowed, and passed in the feces (Comolli and Divers 2021). In squamates, the pulmonary lumen is large, and the tracheal diameter may be smaller than that of the pentastomids. That poses a risk of respiratory obstruction by the adult pentastomids. However, the greatest pathological changes are seen in the intermediate host, where they may become embedded in tissues (Wellehan and Walden 2019).

Pentastomiasis in reptiles, especially snakes, occurs subclinically. Although the infection is large, death is usually associated with secondary septicemia and pneumonia (Tappe and Büttner 2009). Because pentastomids attach to the lung tissue, they can cause perforation, hemorrhaging, and serious inflammation in the host (Fernando and Fernando 2014). In a Common Garter Snake (*Thamnophis sirtalis* Linnaeus 1758), lung lesions were associated with one of the pentastomida species, *Kiricephalus coarctatus* Diesing 1850, where the embedded parasite had gotten into the hypaxial muscle. Infections with *Armillifer armillatus* Wyman 1845 have also been linked to parasitic pneumonia in gaboon vipers. Pentastomids occluding the trachea have been implicated in snake deaths. Bacterial infections could develop due to lung damage. For example, there are four out of nine *Boa constrictors* with pulmonary damage from *Porocephalus dominicana* Riley & Walters 1980 infection perished due to bacteremia and pericarditis (Walden et al. 2021).

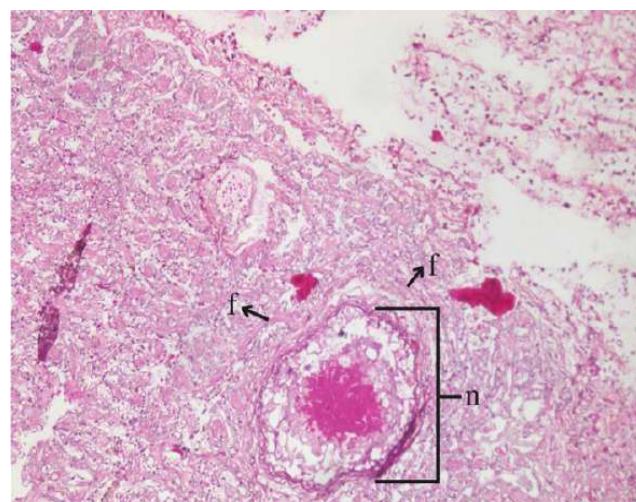


Figure 5. Histological microscopic picture of nymph-infected pulmo from pentastomida showed the presence of fibroblast around the area of infection. f: fibroblast, n: the body of the nymph stage pentastomida

Bigger implications regarding possible impacts on human health may be anticipated based on the histopathological changes found in this study. Humans are the final hosts of pentastomic, which can be contracted from improperly cooked meat from infected snakes or drinking water contaminated with pentastomida eggs. In addition, humans can get the infection when handling and harvesting snakeskin or having direct contact with the mouth of a pet snake (Latif et al. 2016). The pathological consequences of the clinical symptoms experienced by infected humans may be the same or even more severe. That depends on the number of parasite eggs ingested, the duration from the larvae migrating to various organs to maturity and reproduction, and how severe the parasite infestation is in the human viscera.

Personal hygiene measures are required to prevent human infections. For example, avoid drinking river water directly or boiling the water before drinking. In addition, authorities should provide health education to warn people about eating undercooked snake meat, the possibility of transmission after handling snakes, and avoiding contact with snake excretions.

In conclusion the prevalence of pentastomiasis in slaughtered *P. mucosa* in Sidoarjo was 65% (39 of 60 snakes). The pentastomids that infect these snakes are classified as *K. pattoni*. The pulmonary histological findings only show a light reaction in the form of mild fibrosis.

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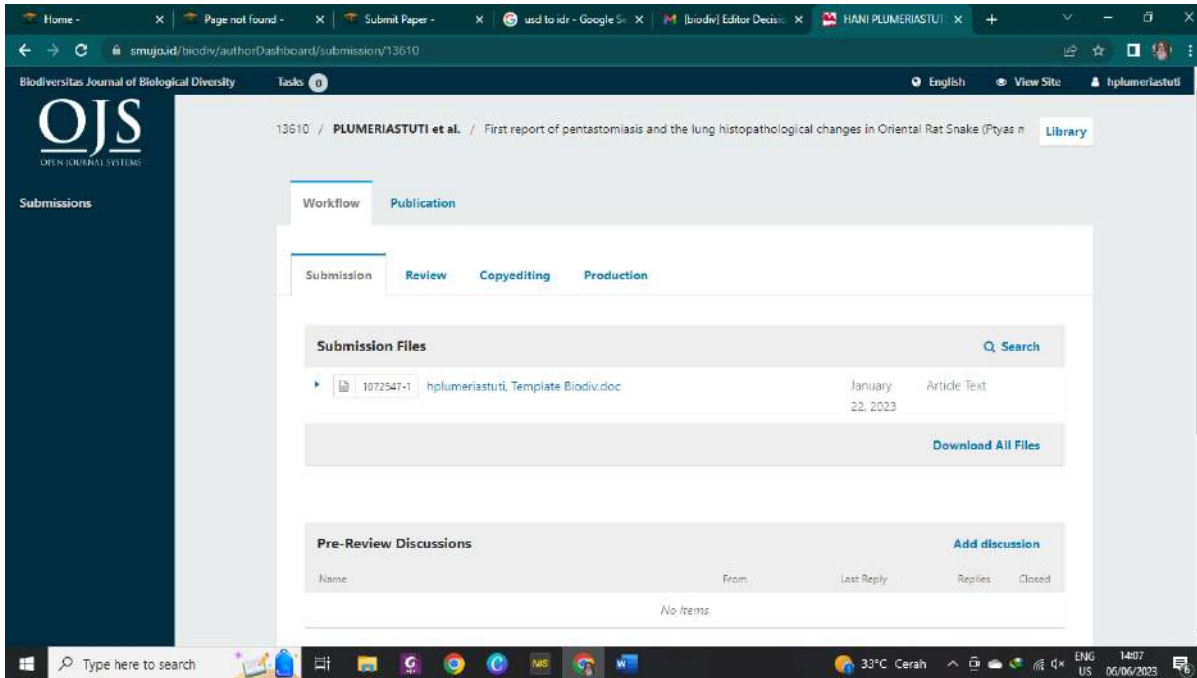
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1. Article Submission



[biodiv] Submission Acknowledgement

External Σ Inbox x



Ahmad Dwi Setyawan <support@mail.smujo.id>
to me ▾

Sun, Jan 22, 11:54 AM ☆ ↶ ⋮

Hani Plumeriastuti:

Thank you for submitting the manuscript, "First Report of Pentastomiasis in *Ptyas mucosa* in Sidoarjo, East Java, Indonesia, and the Lung Histopathological Changes" to **Biodiversitas Journal** of Biological Diversity. With the online **journal** management system that we are using, you will be able to track its progress through the editorial process by logging in to the **journal** web site:

Submission URL: <https://smujo.id/biodiv/authorDashboard/submission/13610>
Username: hplumeriastuti

If you have any questions, please contact me. Thank you for considering this **journal** as a venue for your work.

Ahmad Dwi Setyawan

Biodiversitas Journal of Biological Diversity

2. Article Review

Biodiversitas Journal of Biological Diversity Tasks 0 English View Site hplumeriastuti

13610 / PLUMERIASTUTI et al. / First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (Ptyas mucosa) in Sidoarjo, East Java, I Library

Workflow Publication

Submission Review Copyediting Production

Round 1 Round 2 Round 3 Round 4 Round 5

Round 1 Status
New reviews have been submitted and are being considered by the editor.

Notifications

[biodiv] Editor Decision	2023-01-23 05:21 AM
[biodiv] Editor Decision	2023-03-21 12:23 AM
[biodiv] Editor Decision	2023-04-12 07:56 AM

First Revision

Notifications



[biodiv] Editor Decision

2023-01-23 05:21 AM

Hani Plumeriastuti, Garindra Tiara Pranashinta, Lucia Tri Suwanti, Kusnoto, Annise Proboningrat:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "First report of pentastomiasis in Ptyas mucosa in Sidoarjo, East Java, Indonesia, and the lung histopathological changes".

Our decision is: Revisions Required

Reviewer A:

The article which composes 2000-2500 words from introduction to conclusion (table and figure are excluded) will be published as a **short-communication**. If the authors add other data and compose minimum of 3000 words paper from introduction to conclusion (table and figure are excluded), the article will be published as original full-length paper.

-The introduction is too brief, you need to compose about 600-700 words.

-This manuscript has outdated references. At least, you need to compose a minimum 80% of scientific journals published in the last 10 years (2013-2023) and maximum 10% of reference in Indonesian.

-Please write the references based on the author's guidelines, include DOI. Kindly check the author's guidelines here <https://smujo.id/biodiv/guidance-for-author>

e.g:

Kelehear C, Spratt DM, O'Meally D, Shine R. 2014. Pentastomids of wild snakes in the Australian tropics. *Intl J Parasitol Parasites Wildl* 3: 20-31. DOI: 10.1016/j.ijppaw.2013.12.003.

Recommendation: Revisions Required

Second Revision

[biodiv] Editor Decision

2023-03-21 12:23 AM

Hani Plumeriastuti, Garindra Tiara Pranashinta, Lucia Tri Suwanti, Kusnoto, Annise Proboningrat:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "First report of pentastomiasis in *Ptyas mucosa* in Sidoarjo, East Java, Indonesia, and the lung histopathological changes".

Our decision is: Revisions Required

Reviewer A:

Dear Authors,

Thank you for submitting this manuscript that explores pentastomiasis in snakes. This is an interesting and novel findings and has some potential application as a result.

There are some revisions required in order to consider this manuscript for publication. I have included specific feedback on the word document version of the manuscript, please find attached. Make sure that any changes to the manuscript are shown using highlighted text or tracked changes. Additionally, please address the following key areas when making revisions:

1. Methods. Be clear on exactly how and where the snakes were sourced as this could affect disease status. Provide some clearer information on what proportion of the parasites were sampled and whether any other parasites were identified during the study.
2. Watch out for errors in citation and reference style. All citations are currently not formatted to the journal requirements so do revise these.
3. Implications. What are the implications and future direction for this research? Currently the discussion and abstract are very brief and as such they do not summarise the importance of the study's findings.

With these revisions, the work should be in a stronger position overall.

Recommendation: Revisions Required

[biodiv] Editor Decision External Inbox x



Smujo Editors via SMUJO <mail@smujo.id>
to me, Garindra, Lucia, Kusnoto, Annise

Mar 21, 2023, 7:24 AM ☆ ↶ ⋮

Hani Plumeriastuti, Garindra Tiara Pranashinta, Lucia Tri Suwanti, Kusnoto, Annise Proboningrat:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "First report of pentastomiasis in *Ptyas mucosa* in Sidoarjo, East Java, Indonesia, and the lung histopathological changes".

Our decision is: Revisions Required

Reviewer A:

Dear Authors,

Thank you for submitting this manuscript that explores pentastomiasis in snakes. This is an interesting and novel findings and has some potential application as a result.

There are some revisions required in order to consider this manuscript for publication. I have included specific feedback on the word document version of the manuscript, please find attached. Make sure that any changes to the manuscript are shown using highlighted text or tracked changes. Additionally, please address the following key areas when making revisions:

1. Methods. Be clear on exactly how and where the snakes were sourced as this could affect disease status. Provide some clearer information on what proportion of the parasites were sampled and whether any other parasites were identified during the study.

2. Watch out for errors in citation and reference style. All citations are currently not formatted to the journal requirements so do revise these.

3. Implications. What are the implications and future direction for this research? Currently the discussion and abstract are very brief and as such they do not summarise the importance of the study's findings.

With these revisions, the work should be in a stronger position overall.

Recommendation: Revisions Required

Response for Reviewer Comment ✕

Participants [Edit](#)

Smujo Editors (editors)
DEWI NUR PRATIWI (dewinurpratiwi)
Agustina Putri (aputri1)
Hani Plumeriastuti (hplumeriastuti)

Messages

Note	From
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Dear editor, here is the initial manuscript with answers to the reviewer's comments. I have also uploaded the revised manuscript (with highlighted changes) in the revision box.	hplumeriastuti 2023-04-02 06:35 PM
--	---------------------------------------

hplumeriastuti, Answer to Reviewer's Comments.doc

Add Message

Revision for Reviewer A ✕

Participants [Edit](#)

Smujo Editors (editors)
DEWI NUR PRATIWI (dewinurpratiwi)
Agustina Putri (aputri1)
Hani Plumeriastuti (hplumeriastuti)

Messages

Note	From
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Thanks for the corrections and valuable suggestions. We have made revisions according to the corrections from reviewers. We are waiting for further progress on our article. Thank you very much.	hplumeriastuti 2023-04-07 01:45 PM
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Add Message

Third Revision

[biodiv] Editor Decision

External  Inbox 



Smujo Editors via SMUJO <support@smujo.com>
to me, Garindra, Lucia, Kusnoto, Annise ▾

Apr 5, 2023, 9:29 AM   

Hani Plumeriastuti, Garindra Tiara Pranashinta, Lucia Tri Suwanti, Kusnoto, Annise Proboningrat

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity "First report of pentastomiasis in Ptyas mucosa in Sidoarjo, East Java, Indonesia, and the lung histopathological changes".

Our decision is: Revisions Required

Reviewer A:

Recommendation: Accept Submission




Uncorrected Proof

[biodiv] New notification from Biodiversitas Journal of Biological Diversity

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DEWI NUR PRATIWI via SMUJO <support@smujo.com>
to me ▾

Sun, Apr 9, 1:37 PM   

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Link: <https://smujo.id/biodiv/authorDashboard/submission/13610>

Ahmad Dwi Setyawan

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Notifications

[biodiv] Editor Decision

2023-04-12 07:56 AM

HANI PLUMERIASTUTI, GARINDRA TIARA PRANASHINTA, LUCIA TRI SUWANTI, ANNISE PROBONINGRAT, KUSNOTO:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (Ptyas mucosa) in Sidoarjo, East Java, Indonesia".

Our decision is to: Accept Submission

[Biodiversitas Journal of Biological Diversity](#)



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Uncorrected Proof

Participants

Smujo Editors (editors)
DEWI NUR PRATIWI (dewinurpratiwi)
Agustina Putri (aputri1)
Hani Plumeriastuti (hplumeriastuti)

Messages

Note	From
Dear Author(s), Pls. find attached file for an uncorrected proof (Copyedited file). The revised manuscript (Corrected proof) is awaited. Do not worry about layout changes due to revision: our staff will fix it again. Note: Kindly TURN ON track changes when you make improvements.  dewinurpratiwi, Plumeriastuti-Colibrid snake.doc	dewinurpratiwi 2023-04-09 06:37 AM
Thanks for the information on the progress of my manuscript. Please find the corrected proof that I have attached in this discussion column. I will also send proof of payment for the manuscript soon.  hplumeriastuti, 13610-Article-Text-1079305-1-18-20230409 - Corrected Proof.doc	hplumeriastuti 2023-04-09 08:06 AM



Agustina Putri via SMUJO <support@smujo.com>
to me, GARINDRA, LUCIA, ANNISE, KUSNOTO

Wed, Apr 12, 2:56 PM ☆ ↶ ⋮

HANI PLUMERIASTUTI, GARINDRA TIARA PRANASHINTA, LUCIA TRI SUWANTI, ANNISE PROBONINGRAT, KUSNOTO:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (*Ptyas mucosa*) in Sidoarjo, East Java, Indonesia".

Our decision is to: Accept Submission

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3. Payment



DEWI NUR PRATIWI via SMUJO <support@smujo.com>
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Apr 9, 2023, 1:45 PM ☆ ↶ ⋮

You have a new notification from Biodiversitas Journal of Biological Diversity:

You have been added to a discussion titled "BILLING" regarding the submission "First report of pentastomiasis in *Ptyas mucosa* in Sidoarjo, East Java, Indonesia, and the lung histopathological changes".

Link: <https://smujo.id/biodiv/authorDashboard/submission/13610>

Ahmad Dwi Setyawan

...

↶ Reply

↷ Forward

BILLING



Participants

DEWI NUR PRATIWI (dewinurpratiwi)
Hani Plumeriastuti (hplumeriastuti)

Messages

Note	From
Dear Author(s), Kindly find attached an invoice for the publication of your manuscript. dewinurpratiwi_143-HANI PLUMERIASTUTI.pdf	dewinurpratiwi 2023-04-09 06:44 AM
Dear Editor, Please find the attached proof of payment for my manuscript. Thank you very much. hplumeriastuti_Payment Proof.jpg	hplumeriastuti 2023-04-10 04:22 PM
payment received, thank you	dewinurpratiwi 2023-04-12 11:50 AM

4. Copyediting

The screenshot shows the submission interface for Biodiversitas Journal. The top navigation bar includes the journal name, a 'Tasks' indicator, and user information. The main content area is divided into 'Workflow' and 'Publication' tabs. Under 'Publication', there are sub-tabs for 'Submission', 'Review', 'Copyediting', and 'Production'. The 'Copyediting' sub-tab is active, displaying a 'Copyediting Discussions' section with a table that currently has no items. Below this is a 'Copyedited' section with a search icon and a table listing one item: a document titled '1078592-1.aputri1, D240414-Plumeriastuti-Colubrid snake-REV-check.doc' dated April 12, 2023, with an 'Article Text' type.

5. Publication

The screenshot shows the submission interface for Biodiversitas Journal, now in the 'Publication' stage. The 'Status' is 'Published'. A prominent red banner across the top of the main content area states: 'This version has been published and can not be edited.' On the left, a sidebar contains navigation links for 'Title & Abstract', 'Contributors', 'Metadata', 'References', and 'Galley'. The main content area shows the 'Title & Abstract' section. The title is 'First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (Ptyas mucosa) in Sidoarjo, East Java, Indo'. The abstract text is displayed in a rich text editor, starting with 'Abstract. Plumeriastuti H, Pranashinta GT, Suwanti LT, Proboningrat A, Kusnoto. 2023. First report of pentastomiasis and the lung histopathological changes in Oriental Rat Snake (Ptyas mucosa) in Sidoarjo, East Java, Indonesia. Biodiversitas 24: 2043-2057. Currently, reptiles, especially snakes, are increasingly popular as pets worldwide and are often consumed by several communities in Indonesia. This trend needs attention from a public health perspective because it can potentially cause zoonotic diseases, including parasitic disease such as pentastomiasis. This study aimed to determine the prevalence of pentastomiasis, identify the parasite and observe the pulmonary histopathology in Oriental Rat Snakes (Ptyas mucosa Linnaeus 1758) collected in Tulangan, Sidoarjo, Indonesia. The methods used were calculating the prevalence of pentastomiasis, semichem-acetic carmine-staining, and identify the parasite and are based on references on a specimen. Furthermore, histopathological observation and...'. A 'Save' button is located at the bottom right of the main content area.