

Morphology of *Setaria* spp. (Setariidae; Nematoda) in Aceh cattle, Indonesia

MUHAMMAD HANAFIAH^{1,*}, FARIDA ATHAILLAH¹, T. ZAHRIAL HELMI², AMALIA SUTRIANA³

¹Laboratory of Parasitology, Faculty of Veterinary Medicine, Universitas Syiah Kuala. Jl. Teuku Nyak Arief No.441, Kopelma Darussalam, Banda Aceh 23111, Indonesia. Tel./fax. : +62-651-7551536, *email: hanafi_2015@usk.ac.id

²Laboratory of Biochemistry, Faculty of Veterinary Medicine, Universitas Syiah Kuala. Jl. Teuku Nyak Arief No.441, Banda Aceh 23111, Aceh, Indonesia

³Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Syiah Kuala. Jl. Teuku Nyak Arief No.441, Banda Aceh 23111, Aceh, Indonesia

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Abstract. Hanafiah M, Athaillah F, Helmi TZ, Sutriana A. 2023. Morphology of *Setaria* spp. (Setariidae; Nematoda) in Aceh cattle, Indonesia. Biodiversitas 24: 4151-4160. *Setaria* spp. filaria usually inhabit the peritoneal cavity and its microfilaria can be found in cattle blood. Adult worms are considered non-pathogenic but may cause mild fibrinous peritonitis. However, microfilaria can cause serious conditions. The worms migrate randomly to the central nervous system in a non-natural host such as sheep, horses, and goats, causing serious neuropathological symptoms which are known as epizootic cerebrospinal setariosis or nematodiasis cerebrospinal. The objective of this research was to determine the prevalence of *Setaria* spp. microfilaria in the blood of infected Aceh cattle, identify the worm species in the peritoneal cavity, and determine the morphological difference of *Setaria* spp. through light microscope (LM) and Scanning Electron Microscope (SEM). This research was performed on 150 Aceh cattle slaughtered in Banda Aceh slaughterhouse. The hematocrit microcapillary method and modified Knott's Technique were used to determine the presence of *Setaria* spp. microfilaria in blood, while LM and SEM were used to identify *Setaria* spp. adult worm. The results showed that *Setaria* spp. was detected in Aceh cattle blood based on modified Knott's Technique and hematocrit microcapillary method with a similar prevalence of 13.3%. The worm identification revealed the presence of *Setaria* spp. in the peritoneal cavity of Aceh cattle. The morphological differences between *Setaria digitata* and *Setaria labiatopapillosa* were successfully discovered through LM and SEM studies.

Keywords: Lactophenol, nematode, SEM, *Setaria* spp.

INTRODUCTION

Setaria is a nematode in the order Spirurida and the family Setariidae. Adult worms live freely in the abdominal cavity and microfilaria are present in the blood of their definitive host. Different species have different definitive hosts, such as horses for *Setaria equina* and ruminants for *S. digitata* and *S. labiatopapillosa* (Davoodi 2015). The parasite's predilection is in the peritoneal cavity of both domestic and wild animals (Gomez-Puerta and Mayor 2017; Mrifag et al. 2021). The genus *Setaria* is known worldwide as a parasite of domestic and wild mammals, with 43 species have been registered, five of which found in America (Rodrigues et al. 2021).

There is no report on the number and species of *Setaria* spp in Indonesia, this is due to the absence of research conducted until now. In Europe, 6 *Setaria* spp. has been found (Gibson et al. 2014), while in India, 3 *Setaria* spp. (*S. digitata*, *S. cervi*, and *S. labiatopapillosa*) have been reported with infections due to *S. digitata* being more common in bovines (Kumar et al. 2015). The *Setaria* spp. worm parasite shows migrating behavior in deviant hosts, including sheep, goats, and horses (Perumal et al. 2016). The main form of migration in infected horses is ocular migration (Radwan et al. 2016). Goats and sheep that are infected may suffer from cerebrospinal nematodiasis, which causes death and lumbar paralysis.

Siriyasatien et al. (2023) reported that *Anopheles dirus*, *An. peditaeniatus*, and *Armigeres subalbatus* is a vector compment by *S. labiatopapillosa*, *S. digitata*, and *Brugia pahangi*. Setariosis is a fatal threat to vulnerable animals in the tropics country. When a mosquito feeds on an infected host's blood, it will be infected by microfilaria, which will grow into infective larvae (L3) within 2-3 weeks. Mosquitoes that are infected will transmit L3 to vulnerable animals during blood-sucking, and it will later mature in definitive hosts within eight to ten months (Perumal et al. 2016). According to a study by Tamilmahan et al. (2013), setaria larvae usually migrate to other organs and cause pathology lesions. This filaria has been reported can attack the eyes of horses causing blindness (Shin et al. 2017).

One of the obstacles which influence the acceleration of cattle farm development is worm disease in cattle eyes. This disease is indicated by tears coming out of the cow's eyes. When opened the eyelid, a small white thread can be clearly observed moving on top of the eye surface. The cattle will suffer from eye swelling, injury in the cornea, muddiness on the eye cornea, and eventually blindness if the disease progresses. As far as the researcher's experience, the farmers claimed that the worm found in their cattle eye was *Thelazia* spp. However, it turned out that worms migrating into the eyes can also be *Setaria* spp. worms.

Adult *Setaria* worm and its microfilaria are crucial in parasitology research due to the fact that the worm is frequently utilized in diagnostic tests and also as a model

used in laboratory studies on filariasis drugs for humans due to similarities in morphology, histology, and antigens (Perumal et al. 2016). So far, the information regarding the presence of *Setaria* spp. worms have not been reported in Aceh cattle. The objective of the present research is to evaluate the presence and determine the prevalence of *Setaria* spp. microfilaria in the blood of Aceh cattle, to identify worm species in the peritoneal cavity, and to observe the morphological difference of *Setaria* spp. worm using a light microscope (LM) and Scanning Electron Microscope (SEM).

MATERIALS AND METHODS

Study location and period

This research was conducted from February 2022 to September 2022. The *Setaria* spp. worm samples were collected from 150 Aceh cows that were slaughtered in Banda Aceh Slaughterhouse. The worm was transferred into a plastic bag and labeled. The identification of *Setaria* spp. worms were carried out in the Parasitology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh. *Setaria* spp. were put in petri dishes, cleaned, and then cleared using Lactophenol. Scanning Electron Microscope (SEM) analysis was conducted at the National Research and Innovation Agency (BRIN), KST. Soekarno, Cibinong, West Java, Indonesia.

The worm and blood collection

The blood samples were collected from slaughtered cows and were put inside 3 ml vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA, 1-1,5 mg/ml blood). The worm and blood samples were transported to Parasitology Laboratory, Veterinary Faculty, Universitas Syiah Kuala for further analysis.

Microfilaria identification

The modified Knott's technique method

Observation of *Setaria* spp. microfilaria in blood was conducted by Modified Knott's Technique method (Weerasekara et al. 2019; Basude 2023). The blood samples were collected and put inside Ethylene Diamine Tetraacetic acid (EDTA) mixture. Fifteen ml centrifuge tubes were used and around 10 ml formalin 2% was added into 1 ml anticoagulant blood. The tube was turned over several times until the mixture was evenly mixed. The tubes were then centrifuged for 5 minutes at 1000 to 1500 rpm. The supernatant was discarded and a drop of methylene blue stain was added to the tube and mixed using the pipette. A drop of the mixture was put in the middle of an object class, covered, and then examined under the microscope with 10x magnification.

Hematocrit microcapillary method

The examination for *Setaria* spp. microfilaria was also conducted by the hematocrit method using a microhematocrit capillary tube. The blood sample was tested twice (two microcapillary tubes were needed for each). Blood was absorbed by the microcapillary tube until

it reached $\pm \frac{3}{4}$ of the microcapillary tube volume. One of the ends of the microcapillary tube was sealed by crista seal wax. The microcapillary tubes already filled with blood samples were arranged in a microhematocrit centrifuge device and were centrifuged for 2 minutes at 16000 rpm. The hematocrit tubes were examined under the microscope by the buffy coat area (the cluster of white blood cells between plasma and red blood cells in the middle of the tube). If *Setaria* spp. microfilaria is present, the movement could be seen at the buffy coat region. If none of *Setaria* spp. is present, the hematocrit tubes will be broken by the buffy coat area. The buffy coat would be put on object glass, covered by cover glass, and observed under the microscope (Fahrimal et al. 2013; Mathison et al. 2019).

Adult worm identification

The adult worms from the peritoneal cavity of Aceh cow during necropsy were gathered and fixed in AFA solution (93 parts ethyl alcohol 70%, 5 parts formaldehyde, and 2 parts glacial acetate acid), washed by saline, cleared by lactophenol, and examined using a light microscope according to Thwaite (2016). For morphological analysis, samples were dehydrated in ethanol series, clarified by lactophenol, and observed under a light microscope Olympus CX 21. Pictures were taken according to a method explained by Pinheiro et al. (2019) by using a camera microscope HDMI+ USB 1080P/2M SIGMA.

Adult worm identification by SEM

The worm were processed in several steps, which were: Cleaning, performed by submerging the samples in cacodylate buffer (0.2 M sodium cacodylate 42.6 gr + distilled water to 1000 ml with 8.4 pH) for around two hours, agitation in "ultrasonic cleaner" for five minutes (i); Prefixation, samples were put inside 2.5 % glutaraldehyde (5 ml glutaraldehyde + cacodylate buffer up to 40 ml) for 6 hour (ii); Fixation was performed by submerging the samples in 2% tannic acid (2 gr tannic acid in 100 ml cacodylate buffer) for six hours and then washed by cacodylate buffer for 5 minutes four times (iii); Dehydration was performed by submerging the samples in alcohol 50% for 5 minutes 4 times, alcohol 70%, alcohol 85%, and alcohol 95% each for 20 minutes and then by absolute alcohol for 10 minutes 2 times (iv); Drying, samples were soaked in tert-butanol for 10 minutes 2x and then vacuum dried (v); Specimen mounting by attaching samples to specimen stub as needed and stage (vi); Coating the specimen with Au using ion coater. Then specimens were observed under a Jeol JSM-IT200 Scanning Electron Microscope (vii) (Hanafiah et al. 2021).

Data analysis

The data were analyzed descriptively.

RESULTS AND DISCUSSION

The examination of Aceh cattle peritoneal cavity (Figures 1A and 1B) showed *Setaria* spp. (Figure 2) was found in slaughtered in Banda Aceh. The prevalence of the worm is presented in Table 1.

Table 1. The *Setaria* spp. prevalence in Aceh cattle culled in Banda Aceh slaughterhouse

| Number of samples examined | Peritoneal cavity examination of Aceh cattle | | Prevalence (%) |
|----------------------------|--|----------|----------------|
| | Positive | Negative | |
| 150 samples | 70 | 80 | 46.6 |

**Figure 1.** Peritoneal cavity (A) of Aceh cattle (B) was positive for *Setaria* spp. worm**Figure 2.** *Setaria* spp. worm found in the peritoneal cavity of Aceh cattle culled in Banda Aceh slaughterhouse

The prevalence rate of *Setaria* spp. in Aceh cattle was 46.6 %. This result was lower than the data observed by Khedri et al. (2014) found that the overall prevalence of *Setaria* spp. in Sistani and Brahman cattle of Iran were 28.6% and 36.5%, respectively. Out of 148 infected Sistani cattle, 34.4% cattle (51 cattle) were infected with *S. digitata*, 20.9 % (31 cattle) were infected with *S. labiatopapillosa*, 43.9% (65 cattle) showed mixed infection of *S. digitata* and *S. labiatopapillosa* and 0.6% (1 cattle) was infected with mixed infection of *S. labiatopapillosa*, *S. digitata* and *S. marshalli*. At 182 infected Brahman cattle, the respective infection values were 87 (47.8%), 27 (14.8%), 67 (36.8%), and 1 (0.5%). The prevalence of

Setaria in 2-3 years old Sistatni cattle (42.2 %) was higher than in other age categories ($P < 0.05$). In addition, the infection rate between males (25.5%) and females (37.3%) of Iranian Sistani cattle showed a significant difference ($P = 0.009$). Mohanty et al. (2000) stated that the prevalence rate of cattle examined in endemic areas for *Setaria digitata* was 12.5%, with the adult worm in the peritoneum and migrating microfilaria present. Several surveys have been conducted to determine the prevalence of *Setaria* spp. in various regions (North, Northwest, and East) in Iran with the prevalence value ranging between 11.11% to 47% (Khedri et al. 2014; Davoodi 2015).

According to Sundar and D'Souza (2013), setariosis is a polyorganic parasite disease caused by *Setaria* spp. worm. From Aceh cattle peritoneal cavity examination, adult worms were found as presented in Figure 2 which means that the cows suffered from setariosis. These adult worms still could not be differentiated to species level from *Setaria* spp. worm because the morphological difference is still unclear. Thus, other methods to identify species from *Setaria* spp. worm is required. In this research, the morphological difference was determined by the LM and SEM.

Kim et al. (2018) and Gomez-Puerta and Mayor (2017) stated that adult worms are mostly non-pathogenic, although the worm could induce mild fibrinous peritonitis. In the case of ocular infection, the opacity of an eye is observed, which eventually resulting loss of visual function. Lumbar paralysis is a main clinical symptom of central nervous system infection. In the definitive host, these larvae migrate and become adults in the peritoneal cavity. In addition, human infections of *Setaria* spp. microfilaria and adult worm have also been reported in several countries, such as Iran and Rumania (Nabie et al. 2017).

Microfilaria identification using the Modified Knott's Technique method and hematocrit microcapillary method

The examination result of 150 blood samples from Aceh cattle to determine the presence of *Setaria* spp. microfilaria by using Modified Knott's Technique (Weerasekara et al. 2019; Basude 2023) appeared in Table 2, whereas the blood examination of Aceh cattle by hematocrit microcapillary method showed in Table 3 and Figures 3A and 3B. The microfilaria infection rate of *Setaria* spp. in Aceh cattle by Modified Knott's Technique and microhematocrit capillary method showed a similar result, which was 13.3% (Tables 1 and 3). This research obtained the infection rate of microfilaria from *Setaria* spp. worm was slightly higher compared to another research performed by Ngasaman et al. (2021), who found that 2.68% of cattle in the southern part of Thailand were infected with *Setaria* spp. The infection rate found in this research was also higher compared to Alborzi et al. (2020), which observed that 8.76% of cattle blood was positive for *Setaria* spp. using the Knott test.

According to Mrifag et al. (2021), adult female worms can produce microfilaria, which is the infective larvae that are transmitted into the infected host's blood circulation

(microfilariosis). Many *Setaria* species do not perform microfilaria periodicity and only a few microfilariae were observed in the blood. Microfilaria was swallowed by the insect vector during blood feeding, and infective larva (L3) growth occurs in the vector's chest muscle. This microfilaria probably will develop to be infective larvae in mosquito chest muscle within 2-3 weeks. The infected mosquito transmits the infective larvae to other susceptible hosts during their blood meal (Rafee and Amarpal 2016).

Kaur et al. (2015) stated that the larval stage of filaria species usually cannot be differentiated through classic morphology. Genetic marking analysis can be used to accurately differentiate *Setaria* spp. and to determine the genetic diversity between parasites originating from different geographic regions.

Adult worm identification with light microscope

The identification of adult worms found in Aceh cattle peritoneal cavities after they were cleared by lactophenol showed that two species of *Setaria* worms were identified, *S. digitata* (Figure 4) and *S. labiatopapillosa* (Figure 5). The species number found in this study is less than Khedri et al. (2014), Sundar and D'Souza (2015) and Kumar et al. (2015) research.

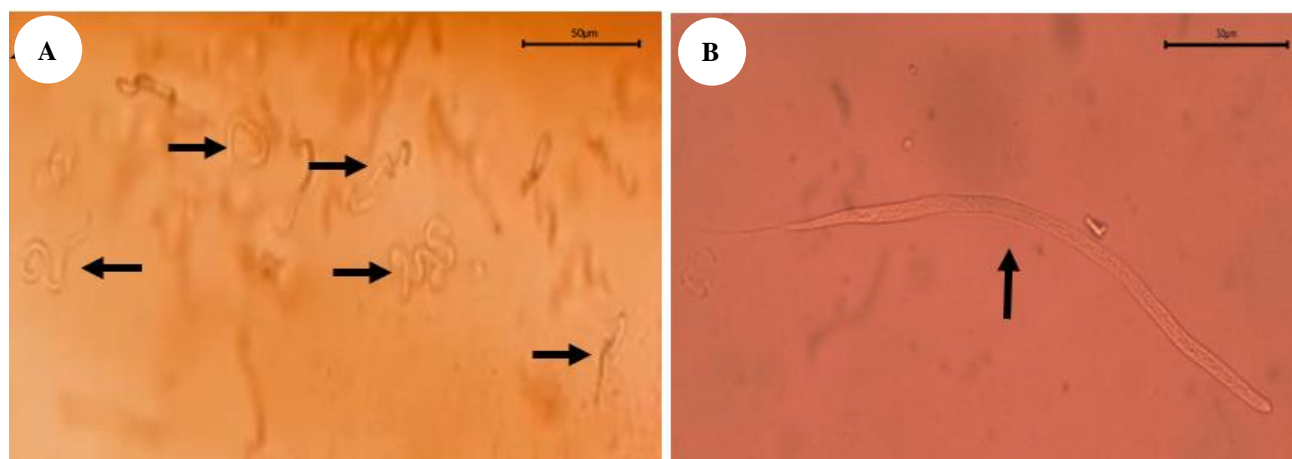


Figure 3. The image of microfilaria of *Setaria* spp. using a hematocrit microcapillary method. A. Several living microfilaria was actively moving; B. One dead microfilaria was shown as a straight line (black arrow). 40x magnification

Table 2. The number of microfilariae in the blood of Aceh cattle found through Modified Knott's Technique

| Number of samples examined | <i>Setaria</i> spp. microfilaria examination | | Infection rate (%) |
|----------------------------|--|----------|--------------------|
| | Positive | Negative | |
| 150 samples | 20 | 130 | 13.3 |

Table 3. The number of microfilaria in the blood of Aceh cattle by using microhematocrit capillary method

| Number of samples tested | <i>Setaria</i> spp. microfilaria examination | | Infection rate (%) |
|--------------------------|--|----------|--------------------|
| | Positive | Negative | |
| 150 samples | 20 | 130 | 13.3 |

Khedri et al. (2014) reported that out of 148 Sistani cattle that were infected with *Setaria*, 51 (34.4%) were infected with *S. digitata*, 31 (20.9%) were infected with *S. labiatopapillosa*, 65 (43.9%) showed mixed infection of *S. digitata* and *S. labiatopapillosa* and one case (0.6%) was infected with mixed infection of *S. labiatopapillosa*, *S. digitata* and *S. marshalli*. Meanwhile Sundar and D'Souza (2015) found that three species *Setaria digitata*, *Setaria cervi* and *Setaria labiatopapillosa* were identified in their. Out of the 500 cattle screened, 187 were found to harbor worms. 56.8 % (106) of animals with *S. digitata*, 24.13 % (45) had *S. cervi* and 18.96 % (36) had *S. labiatopapillosa*.

Kumar et al. (2015) conducted a survey on the occurrence of different species of *Setaria* worms from bovines slaughtered in Chengicherla slaughter house, Hyderabad, Andhra Pradesh, India, and found that among the 148 worms examined, 53% (79), 14.18% (21) and 34.45% (51) were infected with *Setaria digitata*, *S. labiatopapillosa* and *S. cervi*, respectively. The reason for the species variation of *Setaria* spp. in the development of ectopic parasitism is unknown, but there is a possibility that each species of *Setaria* elicits dissimilar immunological responses from the hosts (Shin et al. 2017).

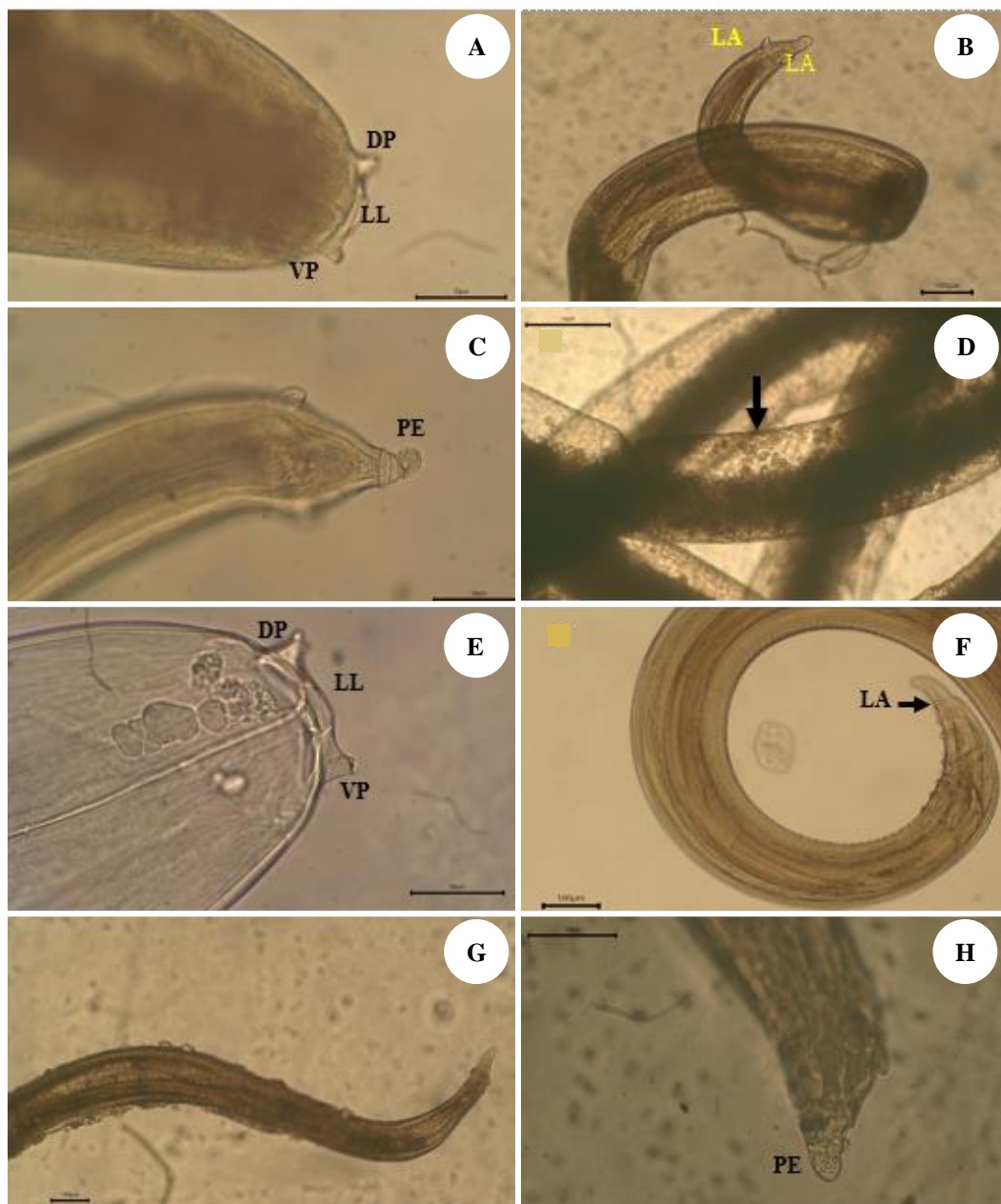


Figure 4. Light microscopic images of adult female *S. digitata*. A. Anterior end of female. B and C. Posterior part with a pair of prominent lateral appendages (LA) and with a smooth knob at the posterior end (PE), D. Black arrow: Uterus. E. Anterior end of male. F. G and H. Posterior end of the male. Abbreviation: Ventral projection (VP), dorsal projection (DP), Lateral lips (LL), Lateral Appendage (LA), and posterior terminal end with smooth knob (PE), S: Spicula. Scale bars: 100 μ m

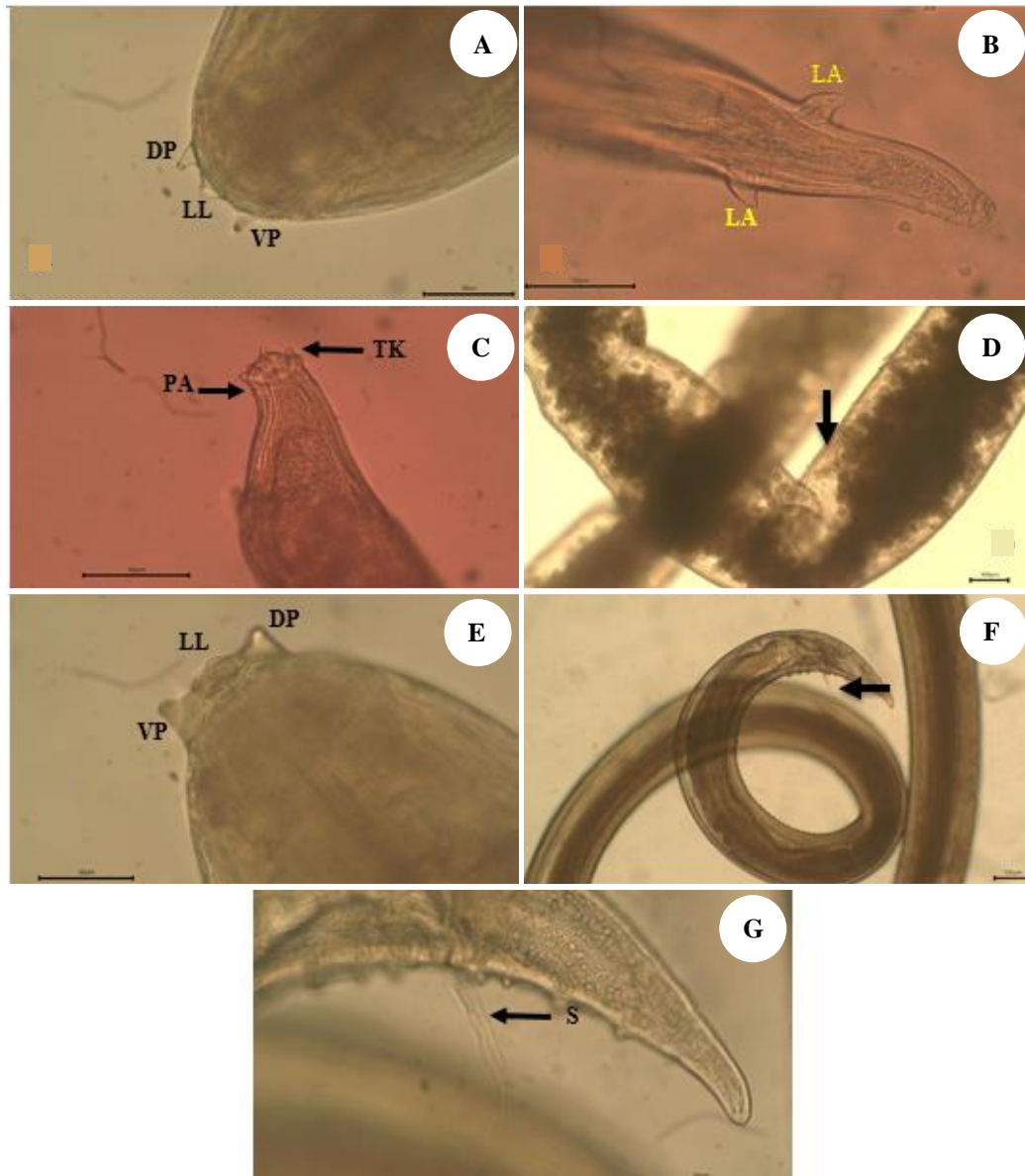


Figure 5. Light microscopic images of adult female *S. labiatopapillosa*: A. Anterior end of female. B and C. Posterior end of the female adult worm. D. Uterus. E. Anterior end of male. F and G. Posterior end of the male. VP: Ventral Projection, DP: Dorsal Projection, LL: Lateral Lips, LA: Lateral Appendage, and posterior terminal end with smooth knob (PE), TK: Terminal Knob, PA: Papilla, S: Spicula. Scale bars: 100 µm

Light microscope images also clearly revealed the morphology of both male and female *S. digitata*. The round lateral lip in both males and females of *S. digitata* was clearly observed. The dorsal and ventral bifid projections of the peribuccal crown were also clearly visible (Figures 4A and 4E). A pair of lateral caudal appendages and a terminal knob were observed at the posterior end of the worm (Figures 4B, 4C, 4G, and 4H) and eggs in the uterus (Figure 4D). The male *S. digitata* showed obvious papillary arrangement, a pair of adcloacal papillae, 3 pairs of post-cloacal papillae, and a central papilla just in front of the cloaca (Figure 4F).

According to Sundar and D'Souza (2015) *Setaria digitata* female worms had a peribuccal crown with a central ‘helmet’ at the cephalic end. The lateral lips were

triangular and the tail end terminated in a smooth knob with oval lateral appendages. In contrast, *S. labiatopapillosa* females had a prominent peri-buccal crown with rectangular lateral lips and the tail end had a smooth button with pointed lateral appendages. Yu et al. (2021) stated that the anterior end of male *S. digitata* was round with two equal prominences on the peribuccal crown and the posterior of female *S. digitata* ended with knob/dull edge that is soft or slightly rough with surface papillae. The anterior of male *S. labiatopapillosa* also had a prominent peribuccal crown and triangular lateral lips, while the posterior end of female *S. labiatopapillosa* has varied spikes (Sundar and D'Souza 2015). Moreover, Singh et al. (2015) the anterior part of the *S. labiatopapillosa* showed a peribuccal chitinous ring with an arrangement resembling

epaulets at the lateral head region. The female posterior end appeared thin, conical, having two lateral appendages, and ended in a structure resembling a rosette carrying several different sizes of nails.

The average body length of *S. digitata* female and male worms was 68.8 ± 3 mm and 45.5 ± 6 mm, respectively. Whereas the average body length of *S. labiatopapillosa* female and male worms was 65.0 ± 3 mm and 35.9 ± 5 mm, respectively.

The *Setaria labiatopapillosa* obtained in this research showed morphological features that are relatively the same where the female worm has a prominent peribuccal crown with rectangular lateral lips (Figure 5A) and the end of its tail has a soft knob with pointy lateral appendages resembling nails (Figures 5B and C) and eggs in the uterus (Figure 5D). The head tip of male *S. labiatopapillosa* also has a prominent peribuccal crown and triangular lateral lip just like in females (Figure 5E). The tail tip of male *S. labiatopapillosa* is very curvy with not so prominent right spicula and the left spicula appears wider in the center and ended unclearly (Figures 5F and 5G).

Some researchers have reported *Setaria* spp. prevalence in cows in several countries (Singh et al. 2015). *Setaria digitata* is generally found in the abdominal cavity of many ruminants, such as sheep, goats, and water buffalo and causes lumbar paralysis (LP) in these ruminants. Afterward, the L3 of this worm may get inside the eye of its definitive host (cattle) and causes blindness or may move to the central nervous system of goats, sheep, or horses through mosquito biting, causing cerebrospinal nematodiasis. Rodrigo et al. (2014) stated that the microfilaria of *S. digitata* has been proven to infect humans, which causes the formation of abscesses, allergic reactions, swelling of lymph nodes, eye lesions, and lung inflammation, which shows gradual adaptation towards humans.

So far, the infection source of both *S. labiatopapillosa* and *S. digitata* found in this research was still unknown, whether they were transmitted by mosquitoes as vectors or from livestock brought into Aceh, Indonesia. According to Azari-Hamidian et al. (2019) *Setaria* spp. is a filaria transmitted through nematode vector spread by mosquito bites and flies (*Haematobia stimulans*), but researchers have yet to study vectors in cattle infected by *Setaria* spp. in Aceh. Thus, further research about the source and factors influencing the incidence risk of *Setaria* spp. should be a common concern. Moreover, further epizootiology observation and entomology study are required to determine setariasis incidence in cattle in Aceh and the deviant hosts, as well as identify the potential of mosquitoes being the vector in the region. The important implications of *Setaria* spp. in animal care and health and its economic impact on the livestock and farm industry should no longer be ignored (Mrifag et al. 2021).

According to Alborzi et al. (2020) cows infected by *S. labiatopapillosa* were 12.3%, while no infection of the worm was found in water buffalo of Iran. The infection prevalence of adult worm, microfilaria, and both were 9.77%, 8.76% and 6.17%, respectively. The prevalence of 437 male cattle was 12.8%, while in females was 11.5%.

Identification of adult worms by SEM

The identification result of adult *Setaria* spp. worm using SEM can be seen in Figures 6 and 7. Several methods have been used in the study of epidemiology and differences in species diversity. Usually, worms are identified based on their morphology, such as cuticle ring, dorsal region, lateral and ventral lips, lateral appendages, opening mouth, spicules, and the cloacal papillae pattern in a male and terminal knob in female worms. Examinations by light microscope have been commonly used to identify various *Setaria* spp. species, however, confirmation of the morphological differences of the worm using SEM is required to produce good quality worm morphology to identify *Setaria* spp. Scanning Electron Microscope is a high-quality tool that can ease the identification process of various morphology in *Setaria* spp. (Kumar et al. 2016).

Based on the examination through a light microscope and SEM on *Setaria* spp. worms confirmed the presence of 2 species: *S. digitata* and *S. labiatopapillosa*. The head of both female and male worms of *S. digitata* showed the roundness uniformity of the buccal opening with adjacent circular lateral lips without any indentations (Kumar et al. 2016), and slightly depressed dorsal and ventral part of the peribuccal crown at the tips (Figures 6A and 6B). The above structures' resolution was improved by many folds in SEM as compared to those resolutions under a light microscope. Longitudinal striations and horizontal bands present on the length of the worm (Figure 6C) only could be clearly observed in SEM but not under the LM. Male *S. digitata* had a coiled spiral end consisting of arranged sexual papillae. Three pairs of pre and post-cloacal and a pair of adcloacal papillae were present at the caudal papillae, and one central papillae were situated in front of the cloaca. A single tongue-shaped spicule was seen projecting from the cloacal orifice (Figure 6C). A pair of small lateral appendages located near the tail tip and terminating into an oval knob (Figure 6D) were in accordance with those reported by Kim et al. (2010). The view of a deirid (Figure 6E) and the postdeirid at the base of which cuticular vertical striations intermit, with the furcated needle-like formation (Figure 6F). Most of the present observations on *S. digitata* were similar with those data reported by Kumar et al. (2016).

On the anterior end of the female *S. labiatopapillosa* peribuccal cuticle ring was found with ventral and dorsal and lips showed curvy elevation, lateral lips (Figure 7A), and mouth opening in an elliptical shape (Figure 7B). Lateral appendages appeared on the posterior end of both sexes (Figures 7C and 7D). Kim et al. (2010) reported that the characteristic *S. labiatopapillosa* species was showed by the presence of dorsal and ventral projections, represented by two summits at the upper terminal end. *Setaria labiatopapillosa* is also characterized by an obtusely ending tail with botryoidal tissue with many spiny projections. Meanwhile, the characteristic of female *S. digitata* showed the lateral lips, represented by one summit. The posterior end of *S. digitata* is a tapering terminus with a smooth knob. The female adult of *S. labiatopapillosa* does not have bifid lateral lips, and the range between the dorsal and ventral projection is smaller than in *S. marshalli*.

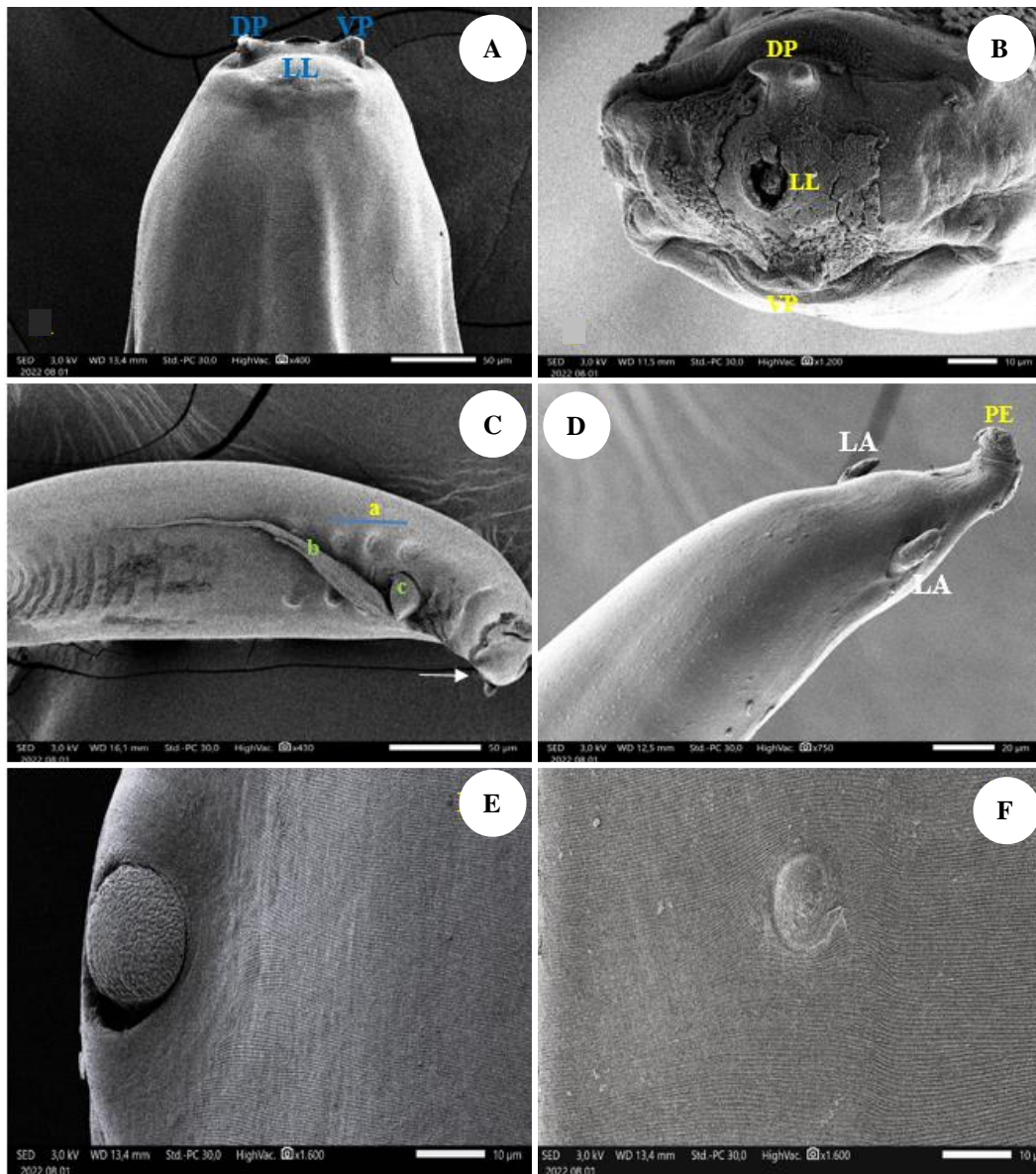


Figure 6. Scanning electron micrograph of *S. digitata*: A. Anterior portion (lateral view), B. Cephalic end (apical view), C. Posterior portion of male (ventral view) showing three pairs of pre-cloacal papillae, D. Posterior portion of female (right lateral view), E. Deirid, a: three pairs of pre-cloacal papillae; b: spicule and c. single papillae; white arrows indicate lateral appendages. F. The postdeirid. Bar: 5 μ m. Note: Dorsal projection (DP), lateral lips (LL), ventral projection (VP), Tapering posterior terminal end of the body with a smooth knob (PE) and lateral appendage (LA)

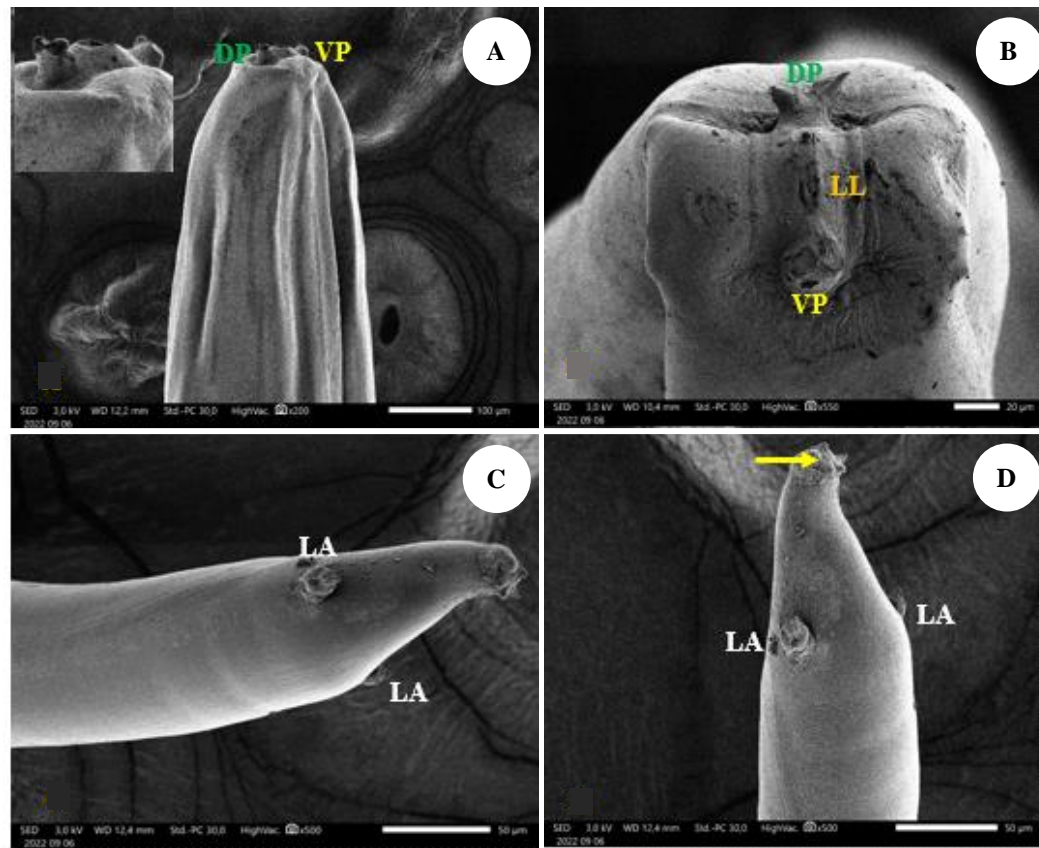


Figure 7. Scanning electron micrograph of the anterior end of *S. labiatopapillosa* female; A. Anterior portion (lateral view), B. Cephalic end (apical view), C and D. posterior end. Dorsal Projection (DP), Lateral Lips (LL), Ventral Projection (VP). E. Deirid

In conclusion, microfilaria of *Setaria* spp. was detected in Aceh cattle using modified Knott's Technique and hematocrit microcapillary method with a similar prevalence of 13.3%. The worm identification revealed the presence of *Setaria* spp. in the peritoneal cavity of Aceh cattle. The morphological differences between *Setaria digitata* and *Setaria labiatopapillosa* were successfully discovered through LM and SEM studies.

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