

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Identification of Acanthocephala Worm in the Small Intestine of Laying Hens from Yogyakarta and Central Java Province, Indonesia

J. Prastowo¹, A. Sahara¹, I. Widayati² and B. Ariyadi³

¹Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta-55281, Indonesia

²Faculty of Animal and Fisheries Science, Universitas Negeri Papua, Indonesia

³Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta-55281, Indonesia

Abstract: This study was conducted to identify the type of Acanthocephala that invaded in the small intestine of laying hens based on morphological examination with Semichon's Carmine staining. A total of 157 laying hens aged over 50 weeks were used. Worms were collected from the intestine, stored in distilled water at 4°C for 2 days and then stained by Semichon's Carmine for worm identification. Nineteen laying hens (12%) were infected by worms and 187 Acanthocephala were collected from posterior part of the small intestine. Morphological observation and measurement of worms were the proboscis, trunk, genital system and eggs. The identified Acanthocephala were creamy white without pseudo segmentation, proboscis shaped like a pear with hooks, spines and divided into anterior and posterior parts. The number of hooks on the anterior proboscis were 18-22 rows, each contains 5 hooks, long cylindrical trunk and tapered at both ends. The trunk had no spine with two long Lemniscus and it was not tied. The reproductive system of male worms comprised of testis, cement glands, vesicles gland and bursa of copulatory. The reproductive system of female worms comprised of the uterus and vagina. Eggs were oval shape with 3 layers of walls and contain embryos. Based on morphological examination of Acanthocephala worms, it was concluded that the species was *Mediorhynchus gallinarum*.

Key words: Acanthocephala, *Mediorhynchus gallinarum*, laying hens

INTRODUCTION

In the Indonesia, industrial poultry farm is one of the most efficient producer of animal protein in which the successful development of livestock laying hens can be affected by various factors such as feed quality, poultry management and disease prevention. Poor poultry management, such as feeding, cage conditions and sanitation can cause hens more susceptible to various diseases. Diseases can be obtained from other animals, the possibility of disease might contact with the intermediate host, or contamination from feed. One of the diseases that often occur due to poor poultry management would be parasitic disease. In Indonesia, poultry farms can not free from parasite infections due to favorable environmental conditions for the parasite. Indonesia's tropical climate could be favorable conditions for the development of worm eggs and survival larvae in nature. Besides, the biodiversity of fauna can provided a vast variety of wildlife species that may become the host for the parasite to complete its life cycle (Satrija *et al.*, 2003).

In the Indonesian poultry farm, one of parasitic diseases was caused by worms, but it often less considered because the slow transmission and low mortality. The parasitic disease can cause economic losses because of stunted growth, weight loss and a decrease in egg

production (Soulsby, 1982; Murtidjo, 1992). Worm infections in the gastrointestinal tract of laying hens that have been reported including *Ascaridia galli*, *Heterakis gallinarum*, *Raillietina* spp and *Echinostoma revolutum*. In the intestinal chicken, Acanthocephala have been reported in various countries such as the Philippines, Papua New Guinea and even Indonesia. In the Indonesia, the reports on the Acanthocephala invasion in laying hens were still very limited. Morphological examination of Acanthocephala with Semichon's Carmine staining never been done. Therefore, this study was conducted to identify the type of Acanthocephala that invaded in the small intestine of laying hens based on morphological examination with Semichon's Carmine staining.

MATERIALS AND METHODS

Experimental birds: A total of 157 laying hens from the farm and the slaughterhouse in Yogyakarta and Central Java province was examined. Thirty laying chickens were collected from Kulon Progo district, 35 laying chickens were collected from the Sleman district, 20 laying chickens were collected from Magelang district and 72 laying chickens were collected from Klaten district. Collected laying hens were slaughtered and the intestine were collected. The number of infected chickens and the number of worms were counted.

Identification of acanthocephala worm: Collected Acanthocephala were measured of the length and width of the trunk and then be stored in distilled water at 4°C for 2 days until the proboscis and bursa of copulatory were invagination (Amin *et al.*, 2013b). Worms that to be stained were punctured using a needle and be relaxed between two glass objects and be placed into a dish containing alcohol formalin acetic acid (AFA) and left for 24 hours. Worms were washed with distilled water and be transferred into the series of alcohol from 30, 50 and 70%, respectively for 15 min. Worm were placed in Semichon's Carmine for an hour. The worms were washed by soaking in 70% alcohol for 15 min. Worm then were dropped to 0.5-1.0% HCl-alcohol so that the worm color to be pink and transparent. Worms were dehydrated into the series of alcohol from 80, 95 and 100%, respectively for 15 min. The worm were soaked into xylol for 1 min, then be moved into the object glass and be etched with entellan, then be covered with a glass (Cable, 1977).

Observation of morphological structure of the worms were done in the next day and be conducted under microscope with magnification of 100-400 times. Observations and measurements were morphology of worms including forms of proboscis, the number of hooks on the proboscis, the reproductive organs of male and female worms and the shape and size of worm eggs. Identification of the worm were done by using identification keys of Acanthocephala according to Schmidt and Kuntz (1977) and Amin (1987), then the data were analyzed descriptively.

RESULTS

Figure 1 showed the predilection of Acanthocephala attached to the posterior part of the mucosal small intestine (1a). Macroscopic changes of petechiae in the mucosal small intestine infected by Acanthocephala (1b). Nodules in the mucosal small intestine infected by Acanthocephala (1c).

Figure 2 showed proboscis of Acanthocephala without staining (2a), proboscis of Acanthocephala with Semichon's Carmine staining (2b), adult Acanthocephala (2c). Figure 3 showed male reproductive system of Acanthocephala (3a, b and c), female reproductive system of Acanthocephala (3d), eggs of Acanthocephala (3e). Table 1 showed the measurement of Acanthocephala worms.

DISCUSSION

The results showed that 19 laying hens were infected by Acanthocephala from 4 districts or equal to 12% of the 157 laying hens. About 20% of laying hens were infected by Acanthocephala in Kulon Progo, 13% in Sleman, 20% in Magelang and 5.5% in Klaten district. *M. gallinarum* prevalence have been reported by 24% in seven locations in Papua New Guinea (Talbot, 1971) and the next few years was reported at 2.2 to 42.2% depending

Table 1: Measurement of Acanthocephala worms

Location	Tubangui and Masilungan (1946)	Junker and Boomker (2006)	Amin <i>et al.</i> (2013)
Trunk	Indonesia Without pseudo segmentation	South Africa Pseudosegmentation	Indonesia Without pseudo segmentation
Length of male trunk (mm)	30-50	3-70	8.87-46.25
Width of male trunk (mm)	1.5-2	0.5-2.8	0.87-1.80
Length of female trunk (mm)	65-70	4-110	8-91.25
Width of female trunk (mm)	1.9-2.5	0.6-4	0.95-2.95
Number of anterior spine of proboscis	18 row longitudinal, each 5 spines	18-20 row longitudinal, each 4-5 spines	18-22 row longitudinal, each 5-6 spines
Anterior testis (l x w, mm)	1.85-3.5 x 0.47-1.5	0.37-3.02	0.56-2.50 x 0.17-0.60
Posterior testis (l x w, mm)	0.91-3.2 x 0.45-1.3	0.32-3.01	0.60-2.56 x 0.2-0.62
Eggs (µm)	54.13-69.01 x 39.6-43.02	70-86 x 43-52	47-57 x 24-32

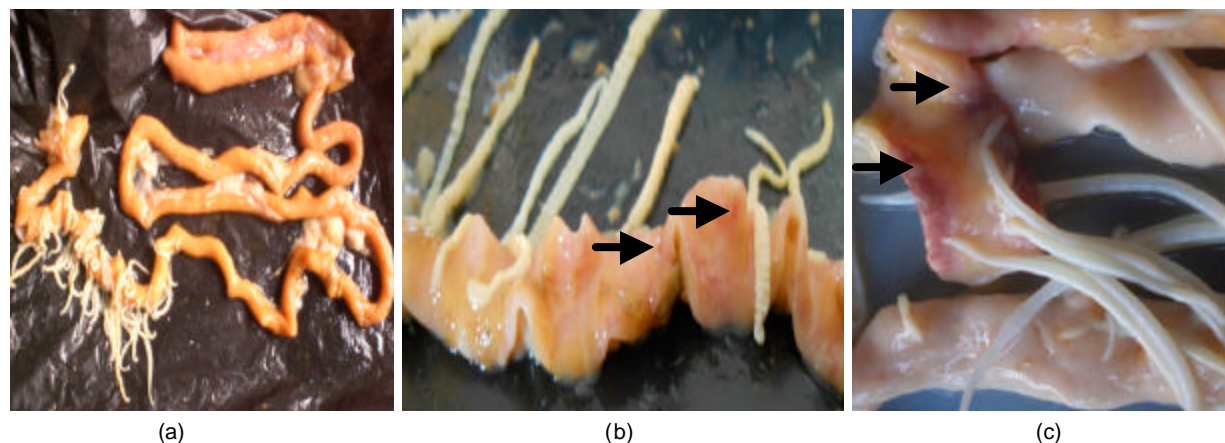


Fig. 1: (a) Predilection of Acanthocephala. The worms attach to the posterior part of the mucosal small intestine, (b) Macroscopic changes of petechiae in the mucosal small intestine infected by Acanthocephala (arrows), (c) Nodules in the mucosal small intestine infected by Acanthocephala (arrows)

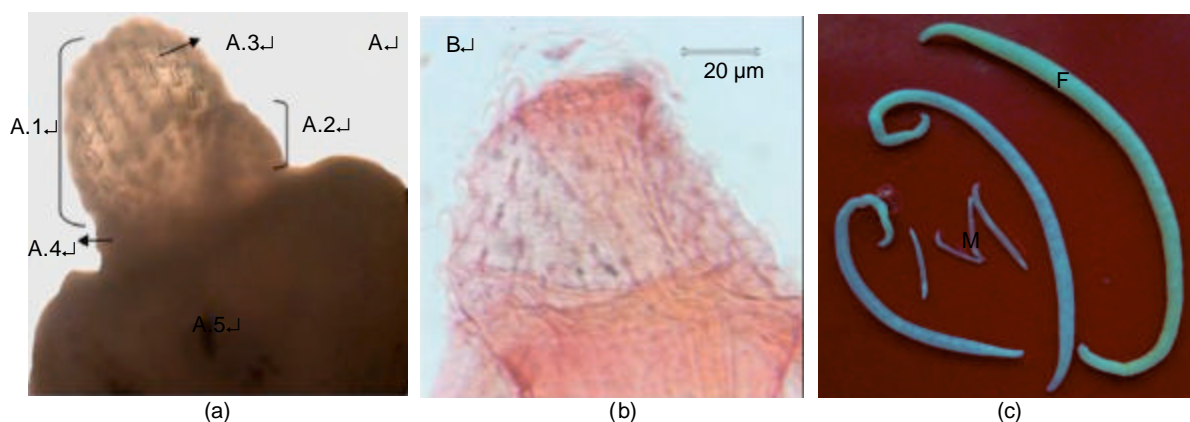


Fig. 2: (a) Proboscis of Acanthocephala without staining, A.1 = anterior proboscis, A.2 = posterior proboscis, A.3 = hook, A.4 = proboscis sheath, A.5 = trunk, (b) Proboscis of Acanthocephala with Semichon's Carmine staining, (c) Adult Acanthocephala, F: Female worm, M: Male worm

on the environmental conditions (Humphrey, 1979). Infections were reported by Amin *et al.* (2013a) in laying hens in Sleman, Yogyakarta, with an incidence of 10-11%, meaning until this research there were increasing of incidence by 1%. Total worms obtained from four districts were 187 Acanthocephala. The number of worms found in the intestines of infected laying hens were at least two worms, while the most one were 51 worms. In the chickens, the intensity of intestinal worms that can cause sub clinical infection seem relatively healthy as non-infected chickens. According to Amin *et al.* (2013a) about 200 worms were found in the intestines can cause clinical symptoms such as diarrhea, loss of appetite, weight loss and unable to walk.

In this study, the observation of intestinal hens showed the predilection of Acanthocephala attached to the

posterior part of the mucosal small intestine. This result supported to Talbot (1971) who found that Acanthocephala in the middle part to the posterior part of the small intestine of local chickens in Papua New Guinea. Petechiae and nodules were found in the mucosal small intestine due to penetration of proboscis. This change was more evident in the chicken intestine infected as much as 51 worm compared to infected only two worms.

Morphology of worms were including a body worms without pseudo segmentation and creamy white color. Female worm structure was greater than male worms. Trunk was long-cylindrical and tapered at both ends. The body wall had no spine. Proboscis had two parts, namely the anterior and posterior equipped with hooks. Anterior proboscis rounded like a pear. Hooks on anterior proboscis had 18-22 longitudinal rows, each

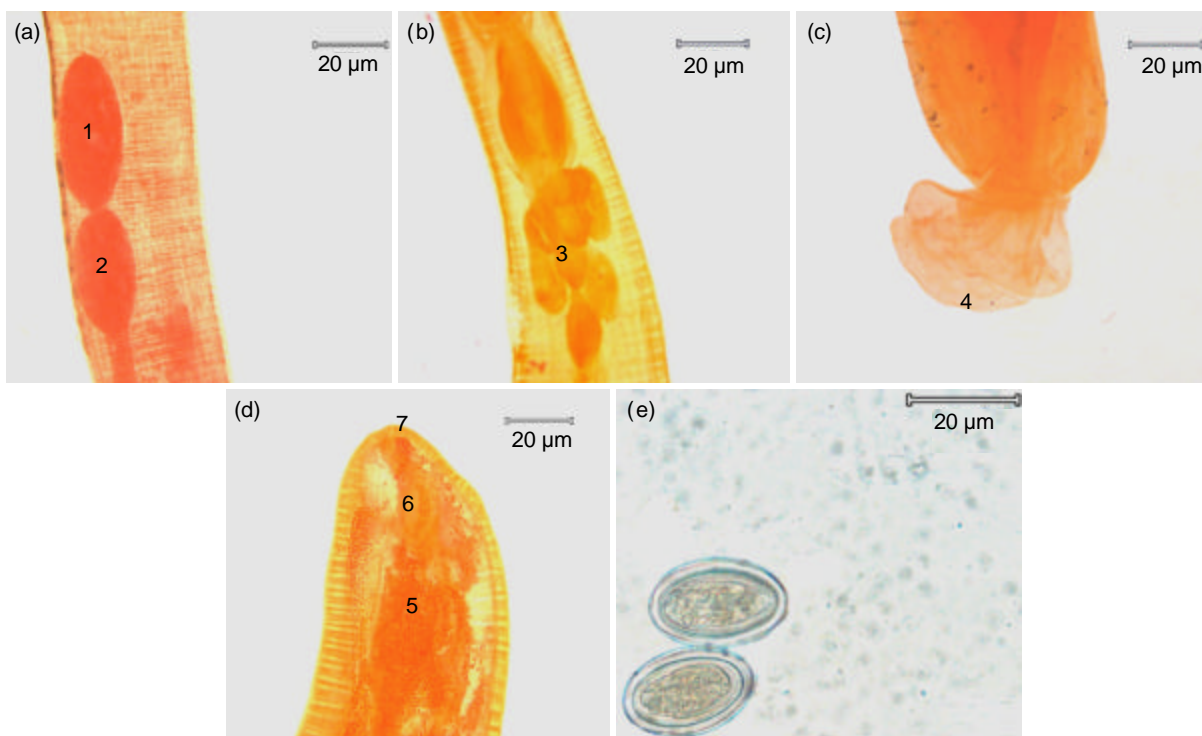


Fig. 3: (a-c) Male reproductive system of Acanthocephala, 1 = anterior testicle, 2 = posterior testicle, 3 = cement glands, 4 = bursa of copulatory, (d) Female reproductive system of Acanthocephala, 5 = uterus, 6 = vagina, 7 = gonopore, (e) eggs of Acanthocephala

consisting of 5-6 hooks with longest hooks located in the middle and towards the posterior. Posterior proboscis were conical shape (extended to the posterior section) with short spines and trunk sections were the zone without thorns. Posterior proboscis have spines shorter than the anterior proboscis so that can be mistaken as a thorn in the trunk when some proboscis were drawn into the proboscis sheath. The worms have single wall in the proboscis sheath, 2 long lemniscus and did not have a neck. These worms have no digestive system and circulatory system, so that nutrients was derived from the intestinal lumen of host through the pores found throughout the body.

Characteristic of male worms were trunk length of 5-90 mm and width of 0.5 to 3 mm. Male reproductive system were located in the posterior of the body consisted of a pair of testicles arranged in the anterior and posterior with varies distance, cement gland, vesicles gland and bursa of copulatory. Anterior testis had length of 1.85 to 3.5 mm and width of 0.47 to 1.5 mm, while the length of the posterior testis was 0.91 to 3.2 mm and width of 0.45 to 1, 3 mm. Cement gland has eight parts and is equipped with a channel.

Characteristic of female worms were trunk length of 25-160 mm and width of 0.7 to 4.2 mm. Female reproductive organs consists of the vagina and the uterus containing worm eggs. Genital orifice of female

worm were located at the posterior end of the body. The produced eggs have 3 layers of walls and contained embryo, oval shape with length of 54.13-69.01 µm and width of 39.6 to 43.02 µm.

Based on the identification key of Amin (1987), Acanthocephala that infects birds might put in Palaeacanthocephala and Archiacanthocephala class. Palaeacanthocephala class often infects the water poultry, while this Acanthocephala infects laying hens. Thus, based on the identification key, this worm might be Archiacanthocephala class that had characteristics of trunk without spine, single wall in the proboscis sheath, oval-shaped eggs with thick walls. The worms might be Gigantorhynchida order, Gigantorhynchidae family, Mediorhynchus genus. This worm had characteristic of rounded shape of proboscis divided into anterior sections with hook and posterior parts with thorns.

In determining the types of Acanthocephala, it can be distinguished based on observation and measurement of morphological characters of the proboscis form, number of anterior and posterior hooks in the proboscis, the neck, the shape of the trunk, the spine on the trunk, pseudo segmentation of trunk, the location of the testicles, the number of cement glands, the position of males and female genital pore and shape and size of the eggs (Monks, 2001).

According to identification key of *Mediorhynchus* species by Schmidt and Kuntz (1977), *Mediorhynchus gallinarum* had characteristics of rounded proboscis (cone like), divided into 2 parts (anterior and posterior), had 80-90 hooks on the proboscis and had pseudo segmentation on the trunk. Junker and Boomker (2006) described that the species had same morphological characteristics with *M. gallinarum* found in the South Africa and Kenya. There are differences in the morphology of the trunk on worms from Asia, including specimens from Indonesia that had no pseudo segmentation and were identified as *M. gallinarum* (Amin *et al.*, 2013a). Based on morphological and molecular characters, Amin *et al.* (2013b) stated that specimens found by Junker and Boomker (2006) and specimens from Africa with pseudo segmentation on the trunk could be identified as *M. africanus*, while *M. gallinarum* without pseudo segmentation were found only from the Asian region. Worms found in this study had no pseudo segmentation in the trunk and had similar morphological characters with specimens found by Tubangui and Masilungan (1946) and Amin *et al.* (2013a).

M. gallinarum could infected small intestine of *Gallus gallus domesticus* as reported by Yamaguti (1954) in Celebes (Sulawesi) and Talbot (1971) in Papua New Guinea. Life cycle of *Mediorhynchus* in birds required the intermediate host as arthropods such as cockroaches. The young worms were located in the small intestine of host, therefore the larval stages were Acanthor, Acanthella and Cystacanth (Moore, 1962; Nickol, 1977; Bolette, 1990). Animals could be infected by feed cockroaches which contain the infective stage of Cystacanth. Larvae then attached in the posterior part of the mucosal small intestine until mature (Nickol, 1977; Bolette, 1990). In the chickens, the clinical symptoms were weight loss, diarrhea and inability to walk. Weight loss might be due to competition in obtaining nutrients between host and parasite in the gastrointestinal tract (Amin *et al.*, 2013a). Macroscopic changes of the mucosal small intestine might be petechiae hemorrhage and damage of the mucosal small intestine (Talbot, 1971).

Geographical distribution of *M. gallinarum* could be broad including in the Asian region. *M. gallinarum* were reportedly found in the India and the Philippines (Tubangui and Masilungan, 1946), in the Celebes (Yamaguti, 1954), in the Terabanon Concepcio'n and Palawan Island (Schmidt and Kuntz, 1977), in the Papua New Guinea (Talbot, 1971; Humphrey, 1979) and in Indonesia (Amin *et al.*, 2013a).

Conclusions: The results showed that the Acanthocephala that infected the small intestine of laying hens might be included Archiacanthocephala class,

Gigantorhynchida order, Gigantorhynchidae family, *Mediorhynchus* genus, *Mediorhynchus gallinarum* species.

REFERENCES

- Amin, O.M., 1987. Key to the families and subfamilies of Acanthocephala, with the erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida). J. Parasitol., 73: 1216-1219.
- Amin, O.M., A.H. Richard, S. Ana and Y. Setyo, 2013a. The Finding of *Mediorhynchus gallinarum* (Acanthocephala: Gigantorhynchidae) in chickens from Indonesia, with expanded description using SEM. Comp. Parasitol., 80: 39-46.
- Amin, O.M., E. Paul, A.H. Richard and M.E. Atif, 2013b. The Description of *Mediorhynchus africanus* sp. (Acanthocephala: Gigantorhynchidae) from galliform birds in Africa. Parasitol. Res., 112: 2897-2906.
- Bolette, D.P., 1990. Intermediate host of *Mediorhynchus orientalis* (Acanthocephala: Gigantorhynchidae). J. Parasitol., 76: 575-577.
- Cable, R.M., 1977. An illustrated laboratory manual of parasitology. Burgess Publishing Company, pp: 265-269.
- Humphrey, J.D., 1979. Helminths of the alimentary tract of the domestic fowl in Papua New Guinea. Aust. Vet. J., 55: 205-207.
- Junker, K. and J. Boomker, 2006. *Mediorhynchus gallinarum* (Acanthocephala: Gigantorhynchidae) in helmeted Guinea fowls, *Numida meleagris*, in the Kruger National Park. S. Afr. J. Vet. Res., 73: 283-292.
- Moore, D.V., 1962. Morphology, life history and development of the Acanthocephalan *Mediorhynchus grandis* van cleave, 1916. J. Parasitol., 48: 76-86.
- Monks, S., 2001. Phylogeny of the Acanthocephala based on morphological characters. Syst. Parasitol., 48: 81-116.
- Murtidjo, 1992. Prevention of poultry disease. Penebar Swadaya, Jakarta, Indonesia.
- Nickol, B.B., 1977. Life history and host specificity of *Mediorhynchus centurorum* Nickol, 1969. (Acanthocephala: Gigantorhynchidae). J. Parasitol., 63: 104-111.
- Satrija, F., Y. Ridwan, E.B. Retnani and R. Tiuria, 2003. The use of anthelmintic for controlling parasitic disease. Proceeding of national seminar. Bogor, pp: 1-7.
- Schmidt, G.D. and R.E. Kuntz, 1977. Revision of *Mediorhynchus* van cleave 1916 (Acanthocephala) with a key to species. J. Parasitol., 63: 500-507.

- Soulsby, E.J.L., 1982. Helminths, arthropods and protozoa of Domesticated Animals. 7th Ed. Philadelphia: Lea and Febiger.
- Talbot, N.T., 1971. An Acanthocephalan parasite, *Mediorhynchus gallinarum*, of the domesticated fowl in Papua and New Guinea. Aust. Vet. J., 47: 334-336.
- Tubangui, M.A. and V.A. Masilungan, 1946. On two Acanthocephala from the Philippines. J. Parasitol., 32: 154-155.
- Yamaguti, S., 1954. Parasitic worms from Celebes. Part 8. Acanthocephala. Acta Medicinæ Okayama, 8: 406-413.