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MORPHOLOGY AND MORPHOMETRY OF *Haemonchus contortus* IN GOATS IN YOGYAKARTA, INDONESIA

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ABSTRACT

This research was carried out to determine the morphology and morphometry of *Haemonchus contortus* in goats. Adult female parasites were collected from abomasum of naturally infected goats and stored in lactophenol. Images of the parasite were acquired using lucida camera and measurement was performed using Axiovision LE software. Basic characters of the morphology and morphometry of *Haemonchus* spp. were identified by determining the value of the discriminant function of three parameters on spicule. Vulvar morphology was identified by vulvar flap shape. Data were presented in terms of mean, standard deviation, and percentage. A hundred percent of the parasites were *H. contortus* with discriminant function value < 0.63. The size of female parasites was longer and wider (25.5-32.6 mm; 0.38-0.63 mm) than that of male ones (17.3-20.0 mm; 0.24-0.33 mm). There were only 2 varieties of vulvar flap, linguiform (81%), and knobbed (19%). There were 5 subclasses identified in the linguiform type, including linguiform A (39%), linguiform B (22%), linguiform C (17%), linguiform I (1%), and a new subclass of linguiform D (2%). Based on the the type of vulvar flap, *H. contortus* found in goats in Yogyakarta were different to those found in previous researches. The findings could be used in identifying the parasite species in small ruminants.

Key words: *Haemonchus contortus*, linguiform D, morfologi, morfometri, vulvar flap

ABSTRAK

Penelitian ini bertujuan mengetahui morfologi dan morfometri H. contortus pada kambing. Parasit betina dewasa diperoleh dari abomasum kambing yang terinfeksi alami dan disimpan dalam laktofenol. Gambar parasit dilakukan dengan bantuan kamera lucida dan pengukuran dilakukan dengan software Axiovision LE untuk karakter dasar morfologi dan morfometri dan identifikasi Haemonchus spp. dilakukan dengan mengetahui nilai discriminant function (DF) dari tiga parameter pada spikula. Determinasi morfologi vulva dibedakan berdasarkan bentuk cuping. Data disajikan dalam bentuk mean dengan standar deviasi dan persentase. Seratus persen parasit adalah H. contortus dengan nilai DF < 0,63. Betina (25,5-32,6 mm; 0,38-0,63 mm) lebih panjang dan lebih lebar dari jantan (17,3-20,0 mm; 0,24-0,33 mm). Hanya ditemukan 2 jenis variasi cuping vulva, yaitu linguiform (81%) dan knobbed (19%). Pada jenis linguiform ditemukan 5 subkelas, yaitu linguiform A (39%), linguiform B (22%), linguiform C (17%), linguiform I (1%) dan subkelas baru, yaitu linguiform D (2%). Berdasarkan morfologi dan morfometri, H. contortus pada kambing di Yogyakarta berbeda dengan hasil penelitian sebelumnya. Hasil penelitian ini dapat digunakan sebagai salah satu cara untuk identifikasi spesies parasit pada ruminansia kecil.

Kata kunci: Haemonchus contortus, linguiform D, morfologi, morfometri, vulvar flap

INTRODUCTION

Gastrointestinal nematode infestation, especially *Haemonchus contortus* (*H. contortus*), was a major problem in goats and sheep health in Indonesia (Haryuningtyas and Artama, 2008). The research conducted in two provinces in Indonesia found that the prevalence of haemonchosis in goats reached 89.4% and the estimated losses resulting from the haemonchosis were a million US dollars per year (FAO, 1991). On the other hand, intensive use of anthelmintics has led to anthelmintic resistance issues. The resistance occurred across the broad spectrum of currently available anthelmintic groups (Max *et al*., 2002; Kaplan, 2004), including in Indonesia (Haryuningtyas *et al*., 2001). Novel developments for the management of nematode parasites such as vaccines, biological anthelmintics, genetic markers and selective breeding of goats may, in the future, provide additional or alternative means of parasite control (Kuchai *et al*., 2012). Precise identification of various species of parasites was crucial in formulating the right strategy of the parasitic control (Amarante, 2011). Spicule morphometry, vulvar flap and cervical papillae were appropriate parameters in the identification of worm species (Rahman and Hamid, 2007). The recent study of the morphology and the morphometry of *H. contortus* was the first attempt made in Yogyakarta.

MATERIALS AND METHODS

Adult female parasites were collected from the abomasum of naturally infected goats slaughtered in abbatoir in the Yogyakarta from June to August 2017. Collection of the parasite from the abomasum was conducted according to Rahman and Hamid (2007). They were stored in a container containing physiological NaCl and transferred into a container containing lactophenol.

Morphology and Morphometry of *H. contortus*

Identification of *Haemonchus* spp. was conducted according to Achi *et al*. (2003) by determining the discriminant function (DF) value of three parameters on the spicule, namely total length (TL), distance between the hook and the tip of right spicule (THr) and the distance between the hook and the tip of left spicule (THl) with the following formula:

$$DF = 0.0016TL + 0.128THr + 0.152THl - 9.97$$

Species of worm was identified following several steps below:

DF < 0.63: *H. contortus*
0.63 < DF < 3: *H. placei*
DF > 4: *H. similis*

Images of parasites were acquired using the lucida camera and measurements were conducted using Axiovision LE software (4.4 release version, Carl Zeiss Vision GmbH, Aalen, Germany) and microscopic photo shooting (Olympus BX51, Tokyo, Japan with Olympus DP12 camera, Tokyo, Japan). The measurement of body length and vulva to posterior end was conducted using vernier caliper under a stereo microscope (Olympus SZ61, Tokyo, Japan). The measurements were conducted for basic characters of morphology and morphometry (Sahai and Deo, 1964; Soulsby, 1982; Kuchai *et al*., 2012; Mir *et al*., 2013).

Determination of Vulvar Morphology

Vulvar morphology was examined under a stereo microscope (Olympus SZ61, Tokyo, Japan) and differentiated by vulvar flap shape into linguiform (with a supra vulvar flap) with 5 morphological variations, namely A (having 1 cuticular inflation), B (without cuticular inflation), C (2 cuticular inflations) and I (cuticular inflation arising from the linguiform process) and D (3 cuticular inflations); knobbed (with knob like vulvar process) or smooth (without any vulvar process) as illustrated by Rose (1966), Le Jambre and Whitlock (1968) and Akkari *et al*. (2013).

Data Analysis

Data were processed using Microsoft Excel and summarized into simple statistics (Kumsa *et al*., 2008). They were presented in terms of mean, standard deviation and percentage.

RESULTS AND DISCUSSION

Based on the DF value, it could be determined that 100% (n: 25) of the parasites used in this study were *H. contortus* with the DF value <0.63. Mono-infection in this result was different from that of Achi *et al*. (2003) and Kumsa *et al*. (2008) suggesting that 51.2% and 38.2% of the examined goat population were double-infected by *H. contortus* and *H. placei* as a result of extensive and traditional breeding method in which the goats and sheep grazed on the same pasture resulting in transmission of infection among them. The goats examined in this research came from semi-intensive farms that raised goats only.

Morphology and Morphometry of *H. contortus*

The parasite had a cylindrical shape, yellowish color with a blunt pointed anterior end. The body of the adult females showed alternating red and white ("barber-pole"), while the posterior tip of the adult males appeared to expand (bursa). Buccal cavity was small and toothed. The size of the females (25.5-32.6 mm; 0.38-0.63 mm) was longer and wider than that of the males (17.3-20.0 mm; 0.24-0.33 mm). The vulva laid approximately 1/5 posterior to the body and was covered with vulvar flap of varying shapes. The bursa consisted of 2 asymmetric lobes and each of them contained 6 rays. The dorsal ray was of the shape of the letter Y. The length of the two spicules was in the range of 0.42-0.47 mm (Table 1 and Figure 1).

The morphological and morphometric features were similar to those of *H. contortus* (Rudolphi, 1802) Cobb 1898 according to Sahai and Deo (1964), Soulsby (1982), Kuchai *et al*. (2012) and Mir *et al*. (2013) based on color, total body length, maximum body width, spicule length, gubernaculum length, male posterior shape, female vulvar flap shape and egg size. In

Table 1. Comparison of *H. contortus* characteristics (Rudolphi 1802) Cobb, 1898

Characters (mm)	Sahai dan Deo (1964)	Soulsby (1982)	Khucai (2012)	Mir (2013)	Present specimens
Body lenght	14-17 (M) 20-27 (F)	10-12 18-30	9.55-11.85 18.38-24.50	15.09-18.72 22.24-26.33	18.57±0.973 28.34±3.967
Max width	0.199-0.265 0.215-0.332	- -	0.15-0.29 0.32-0.64	0.25-0.29 0.39-0.42	0.30±0.025 0.56±0.082
Esophagus	1.444-1.743 1.162-1.662	- -	1.44-2.86 1.29-2.58	2.401 1.899	1.54±0.124 1.85±0.273
Spicules	0.398-0.448	0.46-0.506	0.26-0.52	0.34	0.44±0.018
Gubernaculum	0.199-0.349	-	0.185-0.304	0.253	0.23±0.018
Dist. between post-vulva	3.81-5.31	- 3.06-3.10	2.11-4.45	2.70	5.14±0.729
Dist. between post-anus	0.415-0.513	0.49-0.55	0.35-0.69	0.26	0.56±0.109
Eggs	0.066-0.074 x 0.033-0.049	70-80 x 41-48 µ	0.55-0.95 x 0.3- 0.6	--	0.073 x 0.043± 0.011 x 0.003
Host	Sheep and goats	Domestic animals	Ruminants	Sheep	Goats
Location	India	London	Ladakh	Jammu	Yogyakarta

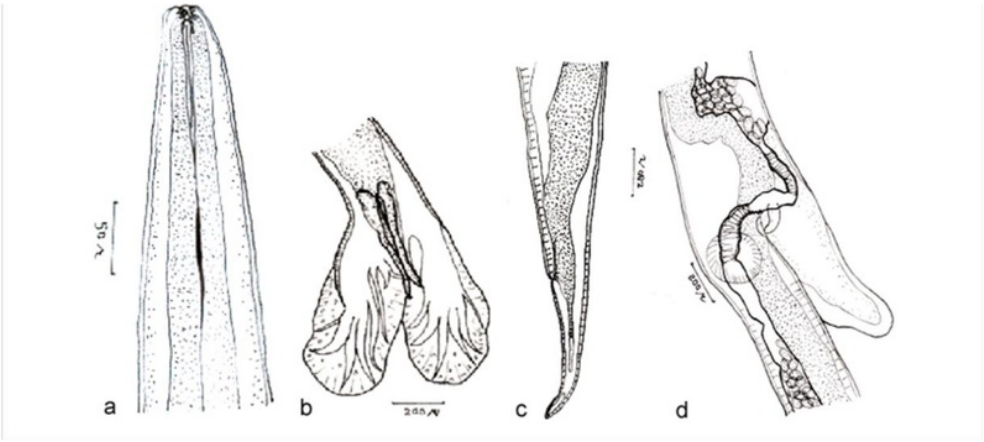

Figure 1. Image of *H. contortus* (Rudolphi, 1802; Cobb, 1898) (drawings were made with the help of lucida camera). A= Anterior end, b= Posterior end of male, c= Posterior end of female, d= Vulvar region of female

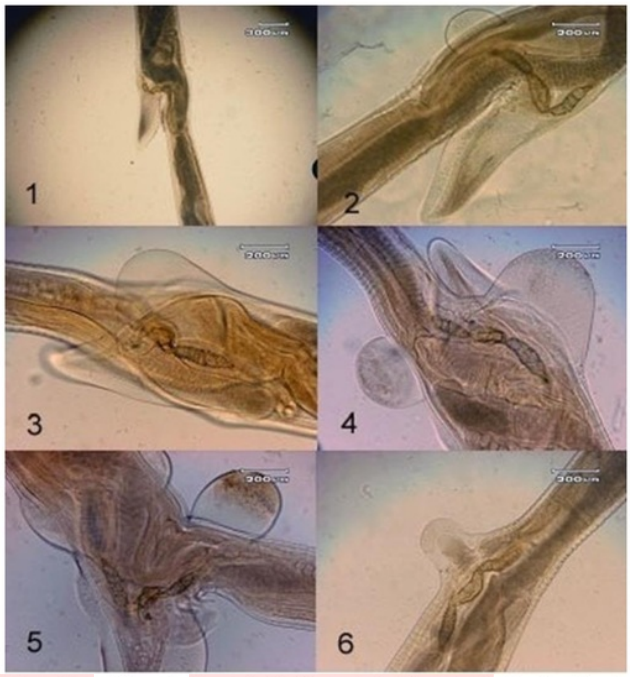

Figure 2. The types of vulvar flap of female *H. contortus* in goats in Yogyakarta. 1= Linguiformis B, 2= Linguiformis A, 3= Linguiformis I, 4= Linguiformis C, 5= Linguiformis D, 6= Knobbed

general, the shape and the size in the research were similar to those found in the previous research.

Determination of Vulvar Morphology

Vulvar morphology of 100 adult females parasites was examined and there were only 2 types of vulvar flap found, namely linguiform (81%) and knobbed (19%) (Figure 2). There were 5 subclasses found in linguiform type, namely linguiform A (39%), linguiform B (22%), linguiform C (17%), linguiform I (1%) and linguiform D (2%) (Figure 2).

The number of the dominant linguiform types was consistent with the findings of Kumsa *et al.* (2008). However, it was highly different from the findings of the project conducted by Akkari *et al.* (2013), Rahman and Hamid (2007) and Gharamah *et al.* (2012) in which the knobbed type was more dominant. Meanwhile, based on the types of the vulvar flap found, the research was different from previous researches in which they found all types of the existing vulvar flap, namely linguiform, knobbed and smooth. Breeding method, geographic condition, climate and parasitic types were considered as the causal factors of the difference in the results of the research. As stated by Akkari *et al.* (2013), the difference in dominance of the vulvar flap type was considered as the result of the variations in agro-ecology, livestock

management, and the genetics of the examined parasites. As already figured out in the findings above, a new type of linguiform (linguiform D) was found in the research as reported by Akkari *et al.* (2013) in which the emergence of this "new species" was considered to relate to *Haemonchus*'s adaptation to the environment. Although the climate in Tunisia (Mediterranean/subtropics) was different from that in Indonesian (tropical climate), we believed that ecological factors played an important role in the changing structure of these nematode parasites. Jacquiet *et al.* (1995) concluded that the vulvar morphology was a marker of the ecological adaptation to certain environment.

CONCLUSION

The *H. contortus* found in goats in Yogyakarta were different to those previously observed in other study based on the types of vulvar flap.

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