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Site distribution and identification of parasitic strongyle from cattle in Central Java, Indonesia

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ABSTRACT

Objective: To identify intestinal strongyle in cattle originated from Central Java.
Methods: Faecal samples from 633 cattle were collected from animals allocated in different areas of Central Java. Samples positive for strongyle type eggs were submitted to coproculture. The L3 strongyle were thereafter isolated and characterized morphologically.
Results: There were 20.4% of the cattle severely infected with strongyles (221 from 633 cattle), 34.4% with moderate and 45.2% with mild infection. The highest total infection of strongyle was found in Sleman. *Cooperia* sp. (32%), *Trichostrongylus* sp. (20%), *Nematodirus* sp. (14%), *Haemonchus* sp. (12%), *Chabertia* sp. (12%), *Oesophagostomum* sp. (6%) and *Ostertagia* sp. (4%) were identified by morphological identification of infective larval stages.
Conclusions: This study shows high incidence of strongylosis in Central Java with different strongyle species observed. The data provide baseline for further investigations of the control strategies for this disease in the region.

1. Introduction

Strongylosis is reportedly a major intestinal helminth parasitosis of cattle worlwide, with consequent substantial economic losses[1]. Manifestation of disease is subclinical or asymptomatic and, therefore, strongylosis is usually chronic[2]. Economic losses are mainly due to decreased body condition score, malabsorption and anemia, possibly complicated with secondary infection such as viral or bacterial infections[3].

In Indonesia, there is a large demand for bovine meat that cannot be suppressed by Indonesia production. The demand is nowadays around 115.932 tons meat, whilst the maximum production is still in 56.029 tons[4]. Presumably, parasitic infections with strongyle are a factor affecting efficiency of meat production due to its prevalence among other intestinal nematodes parasites[5]. The high prevalence of parasites in ruminants is affected by several factors, including the host condition, the parasite and the animal environment. Host-related factors are age, immunity, sex, species and genetic resistance; parasites factors include parasites species, life history, survival larvae in the environment and their location in the host; environmental factors include climate, weather, and season, whereas the interactions between host and parasite environment influence the disease transmission[6]. Environmental conditions, such as temperature and relative humidity are important, besides that, animal husbandry practices such as housing system, deworming intervals and pasture management also play important roles for the onset of strongyle infection[6,7].

Previously, strongyle type nematodes *i.e. Trichostrongylus axei*, *Oesophagostomum radiatum*, *Haemonchus contortus*, *Ostertagia ostertagi* and *Cooperia punctata* were frequently described in Indonesia[8]. *Haemonchus contortus* and *Trichostrongylus* spp. were reported to be the most prevalent and highly pathogenic helminth in livestock[9]. Moreover, *Haemonchus contortus* is the most notorious parasite in livestock due to its biotic potential and blood sucking ability[9].

These worms have a direct life cycle without any intermediate host and therefore accelerate life cycles. After excretion of the eggs, larvae hatch and moult in three stages, *i.e.* larva 1 (L1),

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larva 2 (L2) and larva 3 (L3)[10]. Larvae 1 grow and develop to the second stage larvae which in turn grow and develop into third stage larvae, which are the infective stage and infects animals within one week[10]. Infective larva (L3) lives in grass and forage and if ingested larva develop into fourth stage larvae (L4), thereafter it will be develop in adult stage in intestine of the infected cattle[11]. Identification of the species responsible for strongylosis in ruminants is difficult and commonly hard to perform without recurring to slaughtered animals and examining the adult worms[10,11]. Even though diagnosis by egg examination to recognize the parasite genera could be difficult because of the similarities in the size and appearance of the strongyle eggs, it is possible to observe the size (length and width), the shape and characteristic of the cell to identify the parasite eggs[8,11]. However, the infective larvae present specific characteristics, which are lacking in other free living organism, and allow us to differentiate between species. The infective larvae are usually obtained after coproculture, which offers the environment factors, such as temperature, moisture and oxygenation, necessary for the development of the parasite[12]. The larval culture method is applied to determine the different species of helminthes responsible for the animal infection. Identification of the larvae can strengthen identification of parasites up to species level. This approach can be performed by description of head shape, tail, esophagus types and number of intestine cells[13]. The accurate diagnosis of nematode infections is pivotal for effective control of the parasitosis and can assist substantially in the monitoring of anthelmintic resistance in a strongylid population[12]. Therefore, the present study investigated prevalence of strongyle infections and determined the species of strongyle infecting cattle in Central Java.

2. Materials and methods

2.1. Ethic statement

The procedure on this paper was approved by the committee of ethical clearance for study research of LPPT Gadjah Mada University, Yogyakarta.

2.2. Sample collection

The study was conducted between April and August 2016. In total, 633 faecal samples originated from animals were allocated in specific region of Yogyakarta *i.e.* Sleman, Bantul, Gamping, Gunung Kidul, Kulon Progo, and the region of Central Java *i.e.* Magelang, Muntilan, Prambanan, Surakarta and Boyolali. Faecal samples were collected directly from the rectum then put in sealed containers and stored at 4 ℃ until the examination.

The examinations of faecal samples were performed in the Laboratory of Parasitology, Faculty of Veterinary Medicine, Gadjah Mada University by using both qualitative and quantitative methods. Qualitative flotation method was perfomed with saturated sodium chloride to confirm the presence of parasite eggs[11]. Parasite eggs found were identified based on egg shape, length, width and the characteristic of cell in eggs to classify the strongyle egg[11]. Strongyle egg was identified by oval shape, the egg shell with segmented yolk[14]. McMaster quantitative method was used to determine faecal egg counts [number of eggs per gram (EPG) of faeces][15]. The data were mapped by using Geographical Information Systems[16].

2.3. Coproculture

Faecal samples were thoroughly crumbled before being mixed with sufficient vermiculite, using nonporous stampers, to a depth about 5 cm in wide mouthed glass jars with approximately 1 L capacity. A hole was left in the center of the culture by holding a stamper vertically in the center of the jar. The cultures were moistened sufficiently to ensure that it did not dry out during incubation period but not waterlogged. The jars were incubated in the dark at 26–28 ℃ for 14 days to ensure L3 development. All samples were then processed by Baermann technique by placing the larval culture directly in conical sedimentation glasses filled with tap water and left for 1–2 h. To stimulate larval migration, tap water was first heated until approximately 50 ℃ overnight[17,18].

2.4. Identification of the larvae

Larval suspensions were dropped onto microscope slides. Larvae were stained with Lugol solution (5 g iodine crystals and 10 g potassium iodide in 100 mL distilled water). To preserve the shape of the larvae, 2% formalin was added. Tubes containing larvae were heated gently with bunsen burner. By this step, larvae were dead but in preserved shape and stained well to ease the structure identification. Identification of strongyle larvae based on observation of the characteristic of the anterior part, sheet tail extension (STE) length (the thin shape extension of the sheath caudal), full body length and esophageal type under the light microscope[18].

3. Results

The examination of intestinal strongyle showed 34.9% (221) of the total 633 samples of cattle from Central Java were infected. The highest infected region was Sleman, Yogyakarta with 48 positive samples from 221 (Figure 1), while in Simo, Boyolali, there was no infection (0%). The level of infection was categorized as mild infection (0–200 EPG), moderate infection (200–500 EPG) and severe infection (more than 500 EPG). The research data showed that severe infection (+++) was observed in 20.4% (45/221) of the samples, moderate infection (++) was 34.4%, and mild infection (+) was 45.2%.

Larval identification was conducted to determine the species of strongyle that infected cattle in Central Java. Variations of larvae identified in this study are presented in Figure 2. The result of strongyle larvae identification is shown in Table 1.

Table 1
Identification and measurement of STE (sheath tail extension).

Larva	Mean value* of STE	Percentage
Trichostrongylus sp.	1.20 ± 0.36	20
Ostertagia sp.	2.01 ± 0.03	4
Nematodirus sp.	7.08 ± 0.69	14
Cooperia sp.	2.03 ± 0.43	32
Chabertia sp.	4.13 ± 0.46	12
Haemonchus sp.	2.16 ± 0.21	12
Oesophagostomum sp.	4.89 ± 0.59	6

*: Length of STE L3 divided with length of STE *Trichostrongylus* sp.[13].

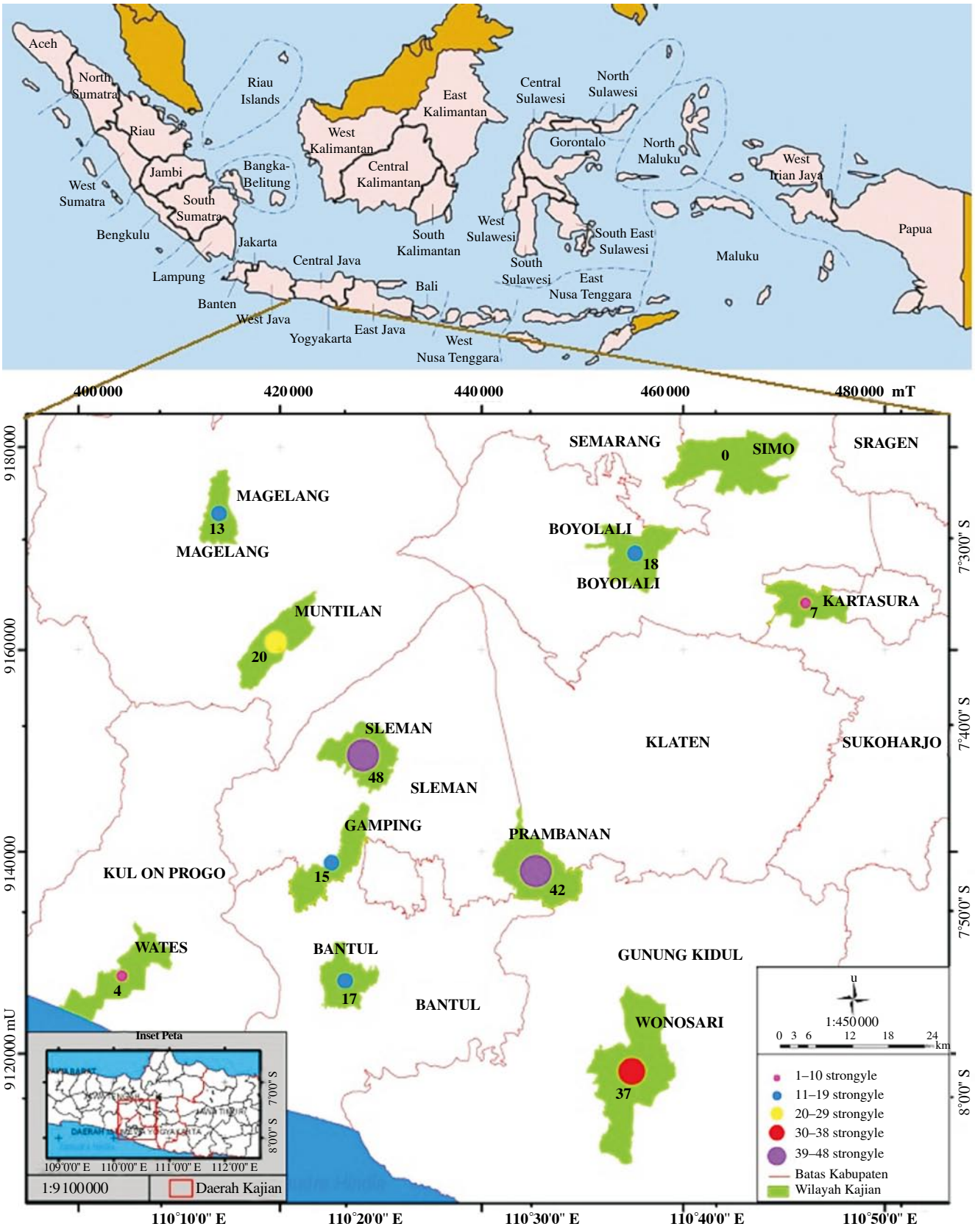

Figure 1. Strongyle abundance and its prevalence in Central Java. Legends represent % infection.

The most prevalent species was *Cooperia* sp. (32%) from the total larvae identified, followed by *Trichostrongylus* sp. (20%), *Nematodirus* sp. (14%), *Haemonchus* sp. and *Chabertia* sp. (12%), *Oesophagostomum* sp. (6%) and *Ostertagia* sp. (4%).

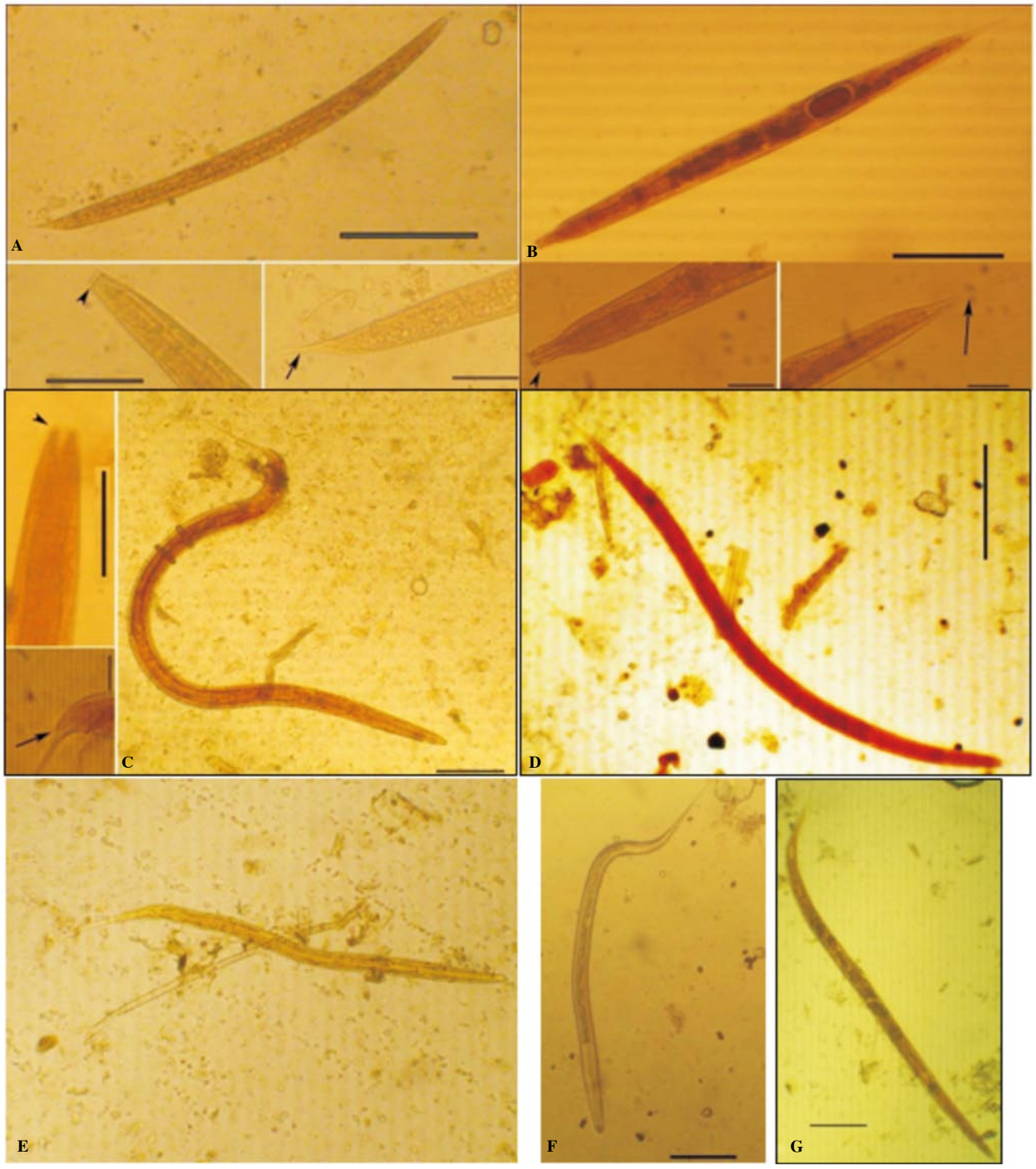

Figure 2. Exemplary figures of larvae examined.
A. *Trichostrongylus* sp.; B. *Ostertagia* sp.; C. *Nematodirus* sp.; D. *Cooperia* sp.; E. *Chabertia* sp.; F. *Haemonchus* sp.; G. *Oesophagostomum* sp. Arrow head is exemplary head of respective larva on the figure panel. Arrows are tail. Scale bars are 100 μm.

4. Discussion

This study shows that cattle in Central Java are highly infected with strongyle parasites. Previous study showed that strongyle infections are the first most prevalent parasite found in cattle[19,20]. Since, strongyles do not need intermediate host in their life cycle, strongylosis spreads rapidly. The diagnosis of strongyle parasites by egg examination is difficult because of the similarities in size, shape, character and appearance.

Among the factors influencing strongyle infections, geographical conditions, temperature, climate, rainfall, humidity, soil conditions and farm management have been described[3,21]. In Indonesia, especially in Central Java Province, tropical climate is dominant throughout the year. High temperature is the most important factor for nematode life cycle and the parasite development from egg to the infective L3 stage[21]. The humidity has been identified as another important parameter for development and survival of strongyle parasites. Rainfall during the wet season increases the humidity and allows the survival of L3 for longer periods in humid soil and pasture and also the complete development to the infective stage[22]. Hence, farm management, particularly cage sanitation and the rotation of pastures are crucial in the control of strongyle infections

by avoiding the completion of the life cycle[13,22]. In Central Java, still the traditional farming system is applied and regular deworming is not practiced. In this region, strongyle infection was considered mainly as mild infection. Although mild infections of strongyle are not harmful to the cattle[22], longer infection can trigger chronic strongylosis, which usually implies as decrease of the body condition score and thus economic losses for the traditional farmers.

Strongyles found in this study were *Cooperia* sp., *Trichostrongylus* sp., *Nematodirus* sp., *Haemonchus* sp., *Chabertia* sp., *Oesophagostomum* sp. and *Ostertagia* sp. The most prevalent species identified in Central Java were *Cooperia* and *Trichostrongylus*. *Cooperia* sp. has ben described as the most economically harmful parasite frequently found in calves and cows small intestine[9]. *Trichostrongylus* species, especially *Trichostrongylus colubriformis* is the most common species in different countries[9]. The STE of *Trichostrongylus* species is without a filament and tapers sharply like a sharpened wooden pencil[13]. *Haemonchus* sp. have the tapered head as a bullet and tapered sheath is short (Figure 2). *Chabertia* sp. and *Oesophagostomum* sp. have similarities and are difficult to be distinguished between them[8,18]. They have a squared head and very long tail as shown in Figure 2. *Nematodirus* sp. have full body longer than other nematode genera and STE (except for *Nematodirus battus*) and the head shape is rounded. Additionally, strongyle parasites are not only harmful to the bovine but also sheep, goat and buffalo. Infections with strongyle have been reported worldwide. In the region of Australia, Malaysia and Brazil, *Haemonchus contortus* were the most pathogenic species[6,9,23]. *Nematodirus battus* were highly found in United Kingdom and *Oesophagostomum* spp. are usually found in Ghana[6,22]. These data show that geographical condition such as temperature and humidity in combination with management system may affect the distribution of strongyle in tropical areas.

This study shows high incidence of strongylosis in Central Java and the variety of strongyle species. Dominant species distributed in Central Java are *Cooperia* sp., *Trichostrongylus* sp., *Nematodirus* sp., *Haemonchus* sp., *Chabertia* sp., *Oesophagostomum* sp. and *Ostertagia* sp. The data may provide baseline for further investigations for improvement of control strategies in the region.

Conflict of interest statement

We declare that we have no conflict of interest.

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