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Major Intestinal Parasites of Domestic Chicken in Boyolali, Central Java, Indonesia

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Abstract
Chicken meat is the largest source of protein in Indonesia and act as an essential factors of food security. However, the chicken farming is frequently hampered by the diseases. Parasitic infections are well-known causing significant economic losses due to drugs, weight loss, inefficient feed conversion rate, drop in egg production and even mortality. The aim of the study was to determine the prevalence of intestinal parasites commonly found in domestic chicken for consumption in Boyolali, Central Java. Two hundred (200) of fecal samples were obtained from slaughtered house in Ngebong market, Boyolali district, Central Java. The chicken population was a mixed between local, meat and layer types. The samples were processed by flotation technique and morphologically identified by light microscopy. Besides, some of post-mortem intestinal tract were also opened to collect adult nematode which were observed colonize in the lumen. Overall prevalence of intestinal parasites reached 55.50% (111/200). The parasites identified were *Eimeria* spp. 28% (31/111), *Ascaridia galli* 6% (7/111), *Capillaria* sp. 25% (28/111), *Railletina* sp. 35% (39/111), *Heterakis gallinarum* 1% (1/111), *Syngamus trachea* 3% (3/111), and *Mediorhynchus gallinarum* 2% (2/111). The high prevalence of intestinal parasites showed that domestic chicken in this area were highly susceptible to parasites infection. The preventive measures were necessary to control poultry parasitosis in the region.

Keywords
Gastrointestinal, parasites, chicken, production

Introduction

Along with the increase of the population in Indonesia, the need to fulfill animal protein also increases [1]. Derived products from chicken are one of the animal food products that are consumed in the largest quantities for both industrial and household needs [2]. Chicken production performance is strongly influenced by genetics, the environment, and the interaction between the two [3]. Besides, environmental factors contribute greatly to the performance of livestock production and reproduction. The environmental factors include the feed provided, maintenance management, and livestock health [2].

Health problems are major constraints of the chicken productivity [4]. Among the diseases, gastrointestinal parasites including worms and protozoa are known as persistent problems. The worms commonly infect the digestive tract of poultry are Nematodes (*Ascaridia* sp., *Heterakis* sp., *Tetrameres* sp., and *Capillaria* sp.), Cestodes (*Railletina* sp.), and Trematodes (*Echinostoma* sp. and *Catatropis* sp.) [5]. Gastrointestinal parasites are parasites that live and eat inside the host gastrointestinal tracts. Parasitic infections have a negative effect on the metabolism of chickens, especially related to the digestive physiology. The presence of worms in small quantities can be tolerated by poultry, but in certain numbers of infection, worms would be detrimental to the health of poultry and interfere with the absorption of feed nutrients [6]. Helminthiasis in chicken hampers feed efficiency and therefore chicken weight

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does not increase although feed consumption remains. The parasitic worms absorb food substances, sucking blood or body fluids, or disintegrate host tissue i.e epithelial villi. Parasitic worms cause damage to intestinal epithelial cells, this therefore reduce the ability of the intestines for absorption of food substances and the production of enzymes in the digestive process [7]. Heavy infestations can cause subsequent drop of egg production, and even mortality [8, 9]. Poultry infected with the parasites are also more susceptible to various diseases and stress [8]. All of the pathogenesis would lead to economic losses and consequence to significant threat to poultry industry in Indonesia. Several diseases in chickens have almost the same clinical symptoms. Therefore, diagnostic approach of causative agent is a principal step in determining strategies to control. In this report, we examined the parasites commonly infect the domestic chicken in the market of Boyolali, Central Java. The report will give an epidemiological data of specific parasites as causative agent that may hampered the chicken production in the region.

Materials and Methods

Fecal samples

The sample collection was carried out from May to June 2022. Samples were taken from a traditional chicken-slaughterhouse at the Ngebong Market, with ordinat 110°22’ - 110°50’ east longitude and 7°7’ - 7°36’ south latitude. The samples were feces from the rectum of slaughtered chicken with the total of 200. Each sample was collected for approximately 4-10 grams. Fecal samples were put onto a sample plastic container, transported to Universitas Sebelas Maret, Indonesia and stored in the refrigerator until observation.

Parasite identification and processing

Fecal samples were checked using the flotation method [10]. Briefly, fecal samples were taken as much as 4 grams and added with 56 ml of tap water. The mixture was then homogenized and centrifuged 4000 rpm, 5 minutes. The supernatant was discarded and pellet was collected. The pellet was vortexed and added with saturated sucrose solution up to 15 ml. The sample was then let in room temperature for 10 minutes. The object glass was placed on the surface of the sample, flipped and covered with a deck glass. Observations were made with a light microscope (Olympus, Japan) to identify the worm eggs or oocysts. The data obtained were then analyzed descriptively compared to relevant literature [11].

The prevalence value of helminthiasis was calculated by the formula as follows:

Prevalence (%) = number of infected samples/number of samples tested x 100%

[12].

Adult parasite staining

Adult parasite obtained i.e *M. gallinarum* was stained by Acetocarmine. Briefly, fixation was performed by pressing the worm between two object glasses and tied with rubber. The fixed worms were then immersed in 10% formalin for 24 hours. After fixation was completed, the worms were washed with tap water and removed from the object glass. The worm specimens were treated in Acetocarmine for 24 hours. Worm specimens were rinsed using tap water, 70% ethanol, and then immersed in glacial acetic acid for 5 minutes. To remove excess color

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without loss of pigmentations, the specimens were dehydrated with ethanol in gradual concentrations. Dehydrations were using 70%, 85%, 95%, and 100% ethanol solutions. Specimens were dipped into each ethanol solution for 5 minutes each. Thereafter, the specimen was soaked in xylene until clear. The stained worms were permanently fixed using Canada balsam and then identified under a light microscope (Olympus, Japan).

Image acquisition

Identification of eggs and oocysts were performed by light microscope. The parasitic objects were acquisited with a microscope camera, Optilab (Miconos, Indonesia). The image processings of scale bars were performed by Image Raster 3.0. (Image Raster Software, USA).

Data analysis

Data were tabulated and graphical presentation were processed by using GraphPad Prism 8.0 software (GraphPad Software, USA).

Results

The 200 fecal samples were obtained from layer, broiler, and local "kampung" chicken. One hundred and eleven (55.5%) fecal samples were positive with at least one parasite (Figure 1.A). Total single infection reached 41% (82/200), co-infection of 2 parasites was 10.5% (21/200), co-infection of 3 parasites was 3.5% (7/200), and 0.5% (1/200) infection by more than 3 parasites (Figure 1.B).

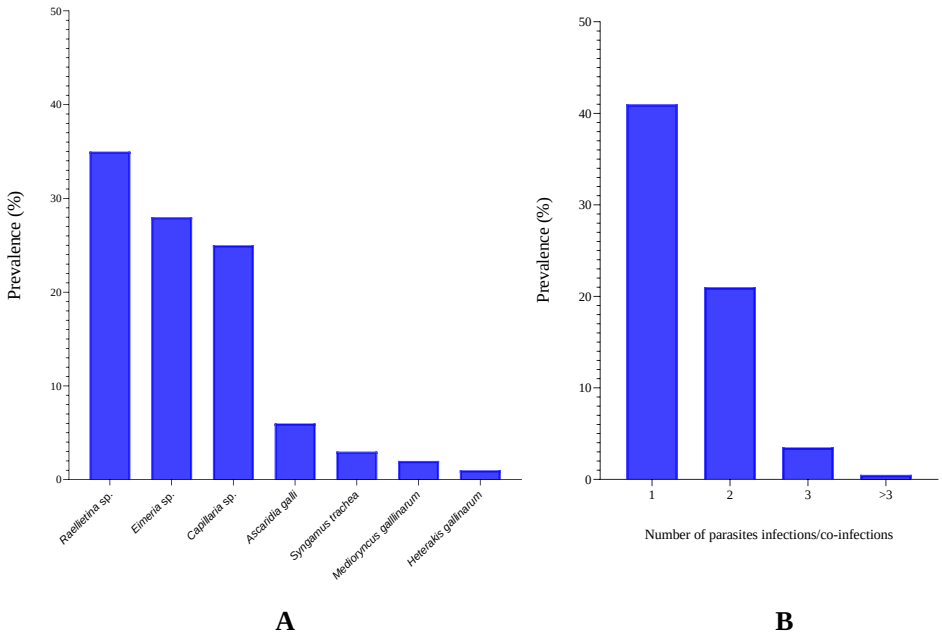

Figure 1. Prevalence of intestinal parasites (A) and co-infection occurred in chicken population (B) from Boyolali District, Central Java, Indonesia

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The results showed that various parasite species were distributed among samples examined (Fig. 1). Parasites found include cestodes, protozoa, and nematodes. Gastrointestinal worms identified were *A. galli* 6% (9/131), *Capillaria* sp. 25% (25/131), *Railletina* sp. 35% (45/131), *H. gallinarum* 1% (1/131), *S. trachea* 3% (5/131), and *M. gallinarum* 2% (2/131) (Fig. 2). The protozoa identified was *Eimeria* spp. (Fig. 3) with a prevalence reached 28% (37/131) (Fig. 1).

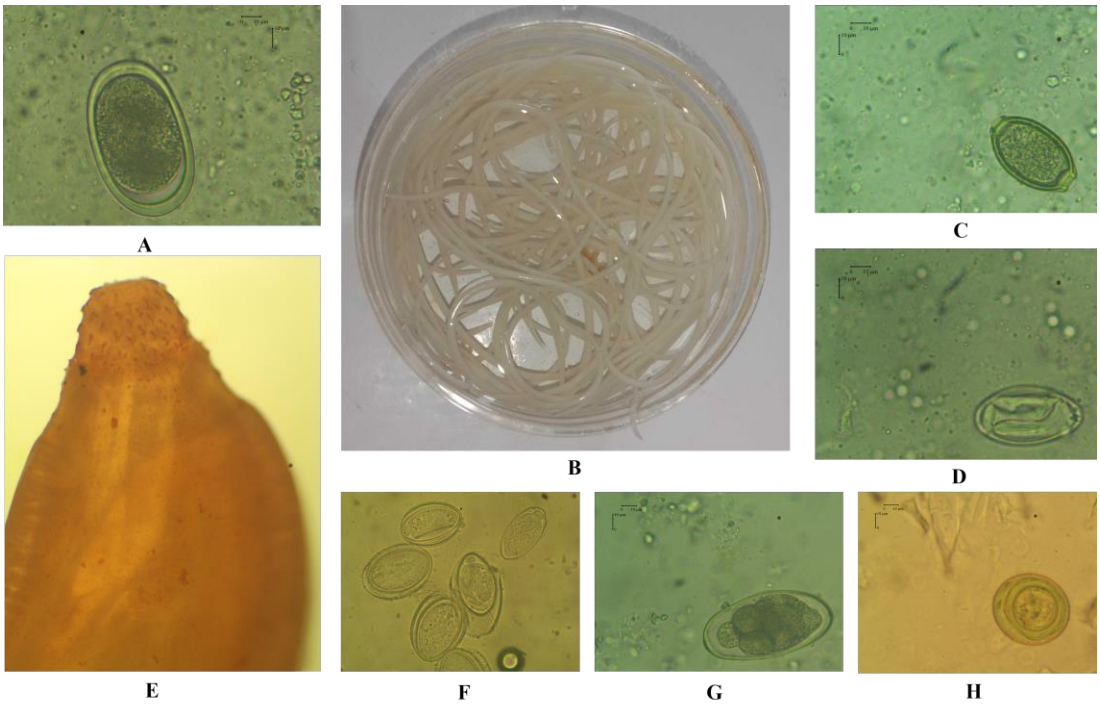

Figure 2. (A) *A. galli* egg, (B) adult *A. galli* (C) *Capillaria* sp., egg(D) *Heterakis gallinarum* egg, (E) adult *Mediorhynchus gallinarum* by Acetocarmine staining, (F) *M. gallinarum* egg, (G) *Syngamus trachea* egg, and (H) *Raillietina* sp. eggs. Scale bars are 10 µm.

Single parasite infection reached 41% (82/200), which were dominated by *Raillietina* sp., 21 % (42/200) and *Eimeria* spp. 14% (28/200) followed by *Capillaria* sp. 5% (10/200), *A. galli* 0.05% (1/200), and *H. gallinarum* 0.05% (1/200) (Fig. 2). Co-infection by 2 parasites reached 10.5% (21/200), by 3 parasites reached 3.5% (7/200), and by more than 3 parasites reached 0.5% (1/200). Chickens were co-infected by two parasites, i.e co-infection of *Eimeria* spp. with *Raillietina* sp. by 4% (8/200), *Eimeria* spp. with *Capillaria* sp. by 1.5% (3/200), *Eimeria* spp. with *H. gallinarum* of 1% (2/200), *Raillietina* sp. with *A. galli* of 1% (2/200), *Raillietina* sp. with *Capillaria* sp. by 2% (4/200), *Raillietina* sp. with *H. gallinarum* of 0.5% (1/200), *S. trachea* with *Raillietina* sp. by 0.5% (1/200). Co-infection by 3 parasites occurred, i.e of *Eimeria* spp., *Raillietina* sp., and *Capillaria* sp. in 3% (6/200) and of *Capillaria* sp., *Raillietina* sp., and *S. trachea* was 0.5% (3/200). Co-infection of more than 3 parasites was found in a sample caused by *Eimeria* spp., *Raillietina* sp., *H. gallinarum*, and *Capillaria* sp.
In this study, gastrointestinal parasites were found in the ova phase which included *A. galli*, *Capillaria* sp., *H. gallinarum*, *S. trachea*, *Raillietina* sp., and *M. gallinarum*. The ova of *A.*

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galli was 80.77 x 48.76 µm ± 0.77 x 1.24 µm, slightly elongated ovals, have a thick wall consisting of 3 distinct layers and was not segmented (Fig. 2.A). The adult phase of *A. galli* worms is milky white and has a length of 7.15 cm ± 0.5 cm. The worm consisted of the anterior part, body, and tail (Fig. 2.B). *Capillaria* sp. ova was 50.55 x 29.4 µm ± 0.55 x 4.4 µm, shaped like a lemon with nearly parallel side walls, non-granulated, non-segmented, and has two plugs at the ends (Fig. 2.C). *H. gallinarum* ova was measured at 54 x 31 µm ± 7 x 1 µm in size. The ova have the same shape as the ova of *A. galli* which were elliptical with a single layer of the wall that is visible but contains larvae inside (Fig. 2.D). *M. gallinarum* was also found in the adult phase. Adult *M. gallinarum* had thick walls, un-pseudo-segmented, rounded, or cone-shaped proboscis anteriorly, and had 80 µm ± 10 µm proboscis hooks (Fig. 2.E). *M. gallinarum* ova were measured at 77 x 54 µm and have 2 layers of thick walls (Fig. 2.F). *S. trachea* ova were measured 86 x 42 µm ± 34 x 25 µm oval shape, thick-walled, and morula with a well-defined wall (Fig. 2.G). *Raillietina* sp. ova were measured at 58 x 55 µm ± 44 x 19 µm, and was observed to have three layers of walls that were thick and distinct, with a characteristic hook in the center (Fig. 2.H).

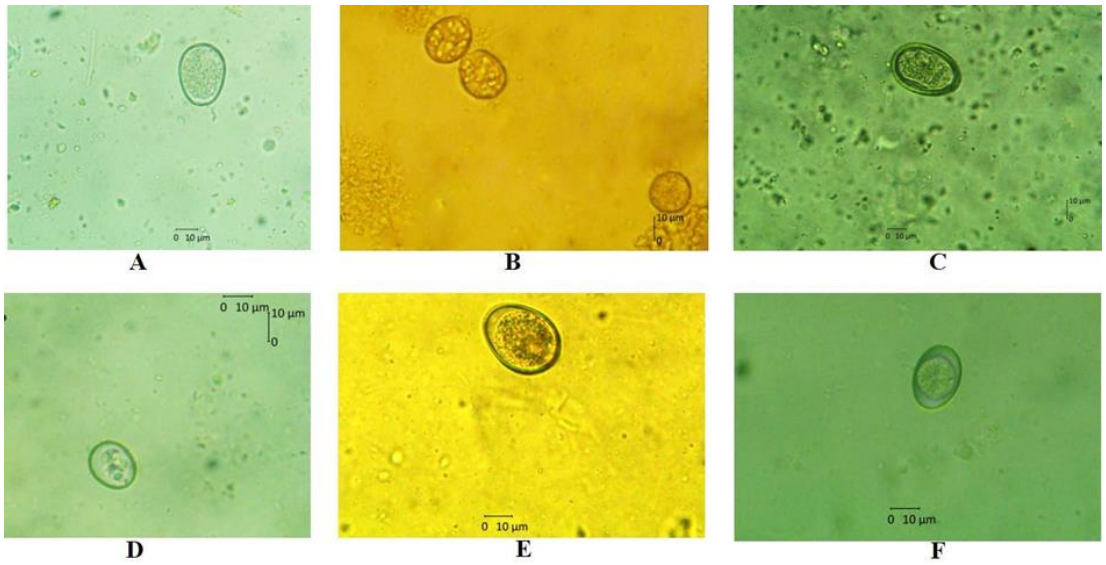

Figure 3. *Eimeria* spp. in various sizes may represent different species circulated in Boyolali, Central Java. Scale bars are 10 µm.

The study results showed *Eimeria* oocysts of various sizes and shapes. Oocysts found were 21 x 17 µm ± 2 x 2 µm (Fig. 3.A), 19 x 18 µm ± 4 x 1 µm (Fig. 3.B), 16 x 14 µm ± 7 x 5 µm (Fig. 3.D), 40 x 28 µm ± 9 x 3 µm (Fig. 3.E), and 16 x 13 µm ± 7 x 6 µm (Fig. 3.F). There were some oocysts in sporulated conditions with characterized by the presence of 4 sporocysts (Fig. 3.B).

Discussions

Chicken farming is frequently hampered by diseases. Parasitic infections are well-known for causing significant economic loss due to drugs for treatment, weight loss, inefficient feed

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conversion rate, drop in egg production, and even mortality [8, 9]. Gastrointestinal parasites prevalence in this study is 55.5% (111/200) including worms at 72% (80/111) and protozoa at 28% (31/111). The prevalence of *Capillaria* sp. and *A. galli* in this study, 25%, and 6%, were higher than the prevalence previously reported in Jember, East Java which were 24.67% and 2.47%, respectively [2]. However, the prevalence of *S. trachea* and *Raillietina* sp. were lower than the study in Lampung [13] and Bangkalan [14]. The *M. gallinarum* prevalence-level is rarely described in investigations in Indonesia. However, it is identified in different geographic areas i.e Sulawesi, Papua, and Sleman [15-17]. In this study, the prevalence of *Eimeria* spp. is lower than an epidemiology survey, which examined a wider area of Central Java [18]. The *Eimeria* spp. species in the current report were not determined since sporulation in potassium dichromate was not performed. The different species presumably occurred since different morphology was documented in this study.

In this study, the highest worm prevalence is the Cestode, *Raillietina* sp., with a prevalence rate of 35%. It is reported that *Raillietina* in Indonesia has reached 60% to 100% in free-range chickens [19]. Parasitic infections of Cestode adversely affect livestock production because the parasite hampers the digestive tract of the chicken [20]. *Raillietina* sp. is the most commonly found in chickens necropsied with damage and hemorrhagic enteritis [10]. *Raillietina* sp. causes degeneration and inflammation of villi in the intestinal mucous membrane since the rostellum hook attachment [20]. Therefore, *Raillietina* can cause reduced growth, emaciation, weakness, and digestive tract obstruction [21]. *Raillietina* sp. requires an arthropods host i.e beetles, soil beetles, black beetles, ants, house flies, and land snails in its life cycle [11]. The chicken of free-range may ingest the intermediate host of *Raillietina* sp. The infestations of Cestoda or Cestodosis in chickens in this report were high since presumably the majority of chicken surveyed was raised in free range husbandry. It is noteworthy, that the high prevalence in free-range chicken can potentially be a source of infection for purebred chickens with modern management that should be a low infection rate.

Various nematode species were also found in this study i.e *S. trachea, A. galli*, *Capillaria* sp., *H. gallinarum*, and *M. gallinarum*. The prevalences of *S. trachea, A. galli*, *H. gallinarum*, and *M. gallinarum* reported here were lower compared to cestode. The nematode species found have different predilection sites of gastrointestinal tract. *S. trachea* infects the upper digestive tract, and the esophagus [22]. The infections cause irritation and inflammatory reactions [23-26]. The *S. trachea* infections in this study is possibly due to contamination of feed from the birds feces infected with the parasite [25, 27]. Subsequent nematode infections were in the small intestine by *A. galli* and *Capillaria* sp. [8]. Adult *A. galli* infections in large numbers cause blockages in the intestine. Thus, it can cause reduced calcium, stunted growth, low productivity, irritation, and inflammation of the mucosa and therefore interfere with the absorption of food [7, 28]. Likewise, *Capillaria* infection causes a decrease in growth rate and weight loss associated with damage to the intestinal mucosa and walls, and increasing succeptibility to secondary infection [8]. Another nematode found, *H. gallinarum*, cause the formation of nodules on the cecal mucosa and granulomas of the liver in severe infection [29-31]. *H. gallinarum* is well-recognized as economically important parasite by poultry industry because its ovum serves as the vector for the other protozoal parasites [32]. The lumen

observations also showed adult worm type of *Acanthochepala*, *M. gallinarum* species. Adult *M. gallinarum* infects parts of the small intestine and large intestine [17]. The proboscis of *M. gallinarum* is able to penetrate the intestinal mucosa causing the formation of white nodules [33, 34]. This nematode infection causes decreased appetite, weight loss, decreased body weight gain, diarrhea, decreased egg production by up to 10%, and inability to chickens for a walk [17]. It is possible for chickens to be infected with this parasite because they eat termites, cockroaches, millipedes, centipedes, and beetles as intermediate hosts [35], as they are raised in free yard traditionally.

The *Eimeria* spp found in this report seemed vary in species. It was reported that 7 *Eimeria* species found in a study in Central Java i.e *E. maxima, E. necatrix, E. acervulina, E. praecox, E. mitis, E. brunetti*, and *E. tenella* [18]. *Eimeria* infection causes coccidiosis which adversely affects the chicken production system [36]. Infected chickens are possible because they eat feed and water contaminated with sporulated oocysts. In Indonesia, coccidiosis is among the most prevalent apicomplexan diseases with huge economic impact in poultry industry.

Our result showed that the infection rate of gastrointestinal parasites was higher than half of the surveyed chicken population which implied that the chickens experienced frequent contact with the source of infection. The feed inefficiency and drug costs cause significant economic losses [9] and therefore may threat for sustainable chicken husbandry [37]. Additionally, the high prevalence of intestinal parasites implied that domestic chickens in this area are highly susceptible to parasites infection. In conclusion, the preventive measures are necessary to control poultry parasitoses in the region.

Conclusion

Gastrointestinal parasites identified in Central Java are *Eimeria* sp. (28%), *A. galli* (6%), *Capillaria* sp. (25%), *Raillietina* sp. (35%), *H. gallinarum* (1%), *S. trachea* (3%), and *M. gallinarum* (2%). The high prevalence of parasitic infestation in chickens implied that the chickens experienced frequent contact with the source of infection and that the domestic chickens in this area are highly susceptible to parasites infection. Our data shows that the preventive and appropriate control efforts need to be performed since the prevalence reached more than half of chicken population examined.

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