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#### **Original Research**





# Identification of Digestive Tract Endoparasites on Laying Hens in Suruhwadang Village, Kademangan District, Blitar Regency

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#### ABSTRACT

This study aims to determine the species, prevalence, and infection degree of endoparasite in the digestive tract of laying hens in Suruhwadang Village, Kademangan District, Blitar Regency. Ninetysix fecal samples were taken from four different farms. Twelve samples of chicken feces aged 20-50 weeks and twelve samples of chicken feces aged >50 weeks were taken from each farm. The fecal examinations in this study are native, sediment, and floating methods. The results of identification of digestive tract endoparasites found consisting of Ascaridia galli (68.75%), Heterakis gallinarum (53.12%), Strongyloides avium (5.21%), Trichostrongylus tenuis (6.25%), Eimeria maxima (51.04%), and Eimeria acervulina (3.12%). The highest average degree of worm egg infection was  $373.96 \pm$ 450.41 found in layers aged >50 weeks, while the highest average degree of infection of protozoa was 296.87  $\pm$  600.92 found in layers aged >50 weeks. The results of the Chi-Square test showed that there was no significant effect of the difference in the age of laying hens in the layer phase on the prevalence of digestive tract endoparasites, both worm egg and protozoan infections (P>0.05). The results of the Mann-Whitney test showed that there was a significant effect of the difference in the age of the laying hen in the layer phase on the degree of worm egg infection (P < 0.05), but the difference in age of the laying hen in the layer phase did not significantly affect the degree of protozoa infection (P>0.05).

#### INTRODUCTION

Laying hens still found to have problems in the digestive tract caused by endoparasites. The impact of these problems is a major loss to the poultry business due to decreased egg production and body weight, malnutrition, and the death of young birds. The economic loss due to gastrointestinal endoparasite infection is estimated to be around 2.49-3.48 million US\$ per year (Hambal *et al.*, 2019).

Common endoparasite infections in poultry include gastrointestinal helminths (cestodes and

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Identification, Infection Degree, Prevalence, Protozoa, Worm Egg.

nematodes) and several species of Eimeria (Puttalakshmamma *et al.*, 2008). A report on helminthiasis infection by Hariani and Simanjuntak (2021) found *Echisnostoma revolutum* (6.67%), *Ascaridia galli* (40%), *Capillaria annulata* (10%), and *Trichostrongylus tenuis* (6.67%). The results of a study by Alifia *et al.* (2023) found the presence of *Ascaridia galli* (66.67%), *Heterakis gallinarum* (45.83%), *Raillietina* sp. (31.25%), and *Strongyloides avium* (7.29%). A coccidiosis case report by Correia *et al.* (2022) described the prevalence of *Eimeria* sp. infection as 13.4%.

Digestive tract endoparasite infections in laying hens are influenced by several risk factors, one of which is farm management (Damayanti et al., 2019). Based on the pre-research survey, the management of layer farms in Suruhwadang Village, Kademangan Subdistrict, Blitar District is still considered poor, especially in terms of cage sanitation. Feces are never cleaned and are seen piling up under the cage. This condition can increase the risk of cestodosis because feces contain organic material for ideal development media for certain insects which may act as intermediate hosts for cestode worms. (Retnani et al., 2009). In addition, there is no research data related to endoparasite infection in the digestive tract of layer-phase laying hens in Suruhwadang Village, Kademangan Sub-District, Blitar District so this research is important to conduct.

#### MATERIALS AND METHODS

This study was a survey study using a crosssectional research design. Ninety-six fecal samples of laying hens were collected from four different farms. Twelve fecal samples of adult (20-50 weeks) and twelve fecal samples of old (>50 weeks) laying hens were taken from each farm. Qualitative fecal examination for the presence of worm eggs and protozoa was conducted using the native, sedimentation, and floating methods. Quantitative fecal examination to determine the degree of infection with worm eggs and protozoa by calculating EPG (Eggs per gram) and OPG (Oocysts per gram) using the Modified McMaster method. Descriptive data analysis was presented in the form of figures and tables. Statistical data analysis was performed using the Chi-Square and Mann-Whitney tests with the SPSS 26 statistical program.

## **RESULTS AND DISCUSSION**

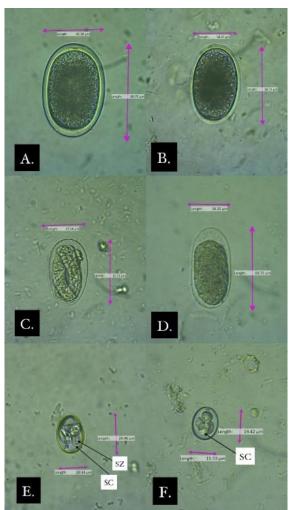
The results of identification and prevalence of endoparasites found in the digestive tract of laying hens include *Ascaridia galli* (68.75%), *Heterakis gallinarum* (53.12%), *Strongyloides avium* (5.21%), *Trichostrongylus tenuis* (6.25%), *Eimeria maxima* (51.04%), and *Eimeria acervulina* (3.12%) which can be seen in Table 1 and Figure 1.

**Table 1.** The Results of Identification andPrevalence of Endoparasites in The Digestive Tractof Layer-Phase Laying Hens in SuruhwadangVillage, Kademangan Sub-District, Blitar District

Species	Prevalence (%)
Ascaridia galli	68.75
Heterakis gallinarum	53.12
Strongyloides avium	5.21
Trichostrongylus tenuis	6.25
Eimeria maxima	51.04
Eimeria acervulina	3.12

The eggs of *A. galli* worms found have an oval shape with smooth walls. The egg wall consists of three layers, namely the inner layer (vitelline membrane), the resistant middle wall layer, and the outer layer (albuminous) (Mubarokah *et al.*, 2019). *A. galli* eggs measure 80.75 x 49.98 µm. The size of *A. galli* eggs found in this study is

by Soulsby's identification key (1982) that *A. galli* eggs measure 73-92 x 45-57 μm.



**Figure 1.** Endoparasites foun in the study with 400x magnification. (A) *A. galli*, (B) *H. gallinarum*, (C) *S. avium*, (D) *T. tenuis*, (E) *E. maxima*, oocysts that have sporulated contain four sporocysts (SC) where each sporocyst contains two sporozoites (SZ) (F) *E. acervulina*, oocysts that have sporulated contain four sporocysts (SC).

The eggs of *H. gallinarum* worms found were elliptical. Eggs have smooth walls and measure  $68.73 \times 38.37 \mu m$ . The size of *H. gallinarum* eggs found in this study is by Soulsby's identification key (1982) that *H. gallinarum* eggs are  $65-80 \times 35-46 \mu m$ . Eggs have a smaller and parallel size when compared to *A. galli* eggs (Riandi *et al.*, 2021).

The Strongyloides avium eggs found have a morphology that is oval with a thin wall. S. avium eggs have a size of  $55.51 \times 37.48 \mu m$ . The size of S. avium eggs found in this study is by Soulsby's identification key (1982) that S. avium eggs measure 52-56 x 36-40  $\mu m$ . Eggs appear transparent and contain larvae (Anupama *et al.*, 2020).

The eggs of *T. tenuis* worms found have a morphology that is oval with a thin egg wall. *T. tenuis* eggs contain 8-32 cells that are undergoing division (blastomere). Eggs have a size of 69.75 x 38.25  $\mu$ m. The size of *T. tenuis* eggs found in this study is by Soulsby's identification key (1982) that *T. tenuis* eggs measure 65-75 x 35-42  $\mu$ m.

Oocysts of *E. maxima* were oval with a large size, slightly yellowish wall, and no micropyle. The size of *E. maxima* oocysts found was 29.90 x 20.34  $\mu$ m. The size of *E. maxima* oocysts found in this study is by the identification key of Soulsby (1982) that *E. maxima* oocysts measure 21.4-42.5 x 16.5-29.8  $\mu$ m. The shape index value (ratio between the length and width of the oocyst) of *E. maxima* in this study was 1.47. This value is by the results of research by\_Silva *et al.* (2022) that the shape index of *E. maxima* is 1.47.

Oocysts of *E. acervulina* were oval with small size, smooth wall, and no micropyle. The size of *E. acervulina* oocysts found was 19.42 x 15.53  $\mu$ m. The size of *E. acervulina* oocysts found in this study is by the identification key of Soulsby (1982) that *E. acervulina* oocysts are 17.7-22.2 x 13.7-16.3  $\mu$ m. The shape index value of *E. acervulina* oocysts in this study is 1.25. This value is by the results of research by Silva *et al.* (2022) that the shape index of *E. acervulina* is 1.25.

The prevalence of *A. galli* eggs in the study was the highest prevalence of endoparasites found. The results of research by Alifia *et al.* (2023) also found that *A. galli* is the most common endoparasite found in the digestive tract of laying hens. The direct route of transmission is one of the factors causing high ascaridiasis in poultry, especially if feed and water are contaminated with infective eggs (Elele *et al.*, 2021).

The prevalence of *H. gallinarum* was found to be higher in the results of Kaufmann *et al.* (2011) at 98%. The higher prevalence value is due to the rearing system of laying hens in the study using a pen system so that the possibility of the host being infected with worms is higher because transmission of *H. gallinarum* worms can occur directly in the environment.

The results of research by Belo *et al.* (2023) found a higher prevalence of *S. avium* at 6.25%. Geographical factors could be one of the reasons because the study location was located at an altitude of 500-1000 meters above sea level, which is higher than the location of this study (BPS Bangli District, 2021). Worm infections in poultry in areas with higher altitudes will have a higher proportion than in areas with lower altitudes because they have higher humidity levels (Permatasari *et al.*, 2020).

The prevalence of *T. tenuis* in this study was categorized as occasional. The grass is one of the factors in the spread of *Trichostrongylus* worm infection because the worm larvae will usually move towards the top of the grass due to light stimulation, especially in the morning and evening (Padilla *et al.*, 2012). Although the locations of the four farms are close to the plantations, they are still limited by the presence of ditches and walls. In addition, the high construction of battery cages can minimize the risk of *T. tenuis* infection in laying hens.

The feces of laying hens that were positive for *Eimeria* sp. infection in this study amounted to 54.16%. The prevalence of *Eimeria* sp. in laying hens was found to be lower in the study of Ybanez *et al.* (2018) which amounted to 43.2%. One of the differences in prevalence can be caused by routine cleaning of feces which is cleaned regularly at intervals of 1-7 days so that cage sanitation is maintained. In this study, feces were never cleaned and appeared to accumulate. This will cause the cage to become more humid and the temperature to increase, creating an optimal environment for the development of oocysts to reach the infective stage (Correia *et al.*, 2022).

The average degree of worm egg infection in laying hens aged 20-50 weeks was  $133.33 \pm$ 118.65. While the average degree of helminth egg infection in laying hens aged >50 weeks was 373.96  $\pm$  450.41. The average degree of protozoan infection in laying hens aged 20-50 weeks is 233.33  $\pm$  767.44. While the average degree of protozoan infection in laying hens aged >50 weeks is 296.87  $\pm$  600.92.

Comparison of the age of laying hens between adult and old hens showed no significant effect on the prevalence of digestive tract endoparasites both infections by worm eggs and protozoa (P>0.05). These results can be observed in Table 2 and Table 3. Adult and old chickens have the same proportion of infection with worm eggs and digestive tract protozoa. This is consistent with the study of Das et al. (2022) that the prevalence of digestive tract endoparasites was found to be high in adult and old chickens compared to young chickens. Adult and old chickens are susceptible to digestive tract parasites compared to young chickens because of the involution of primary lymphoid organs such as the thymus and bursa fabricius which starts from the beginning of the layer phase (20 weeks) to the end of the layer phase which will reduce the production of lymphocytes such as T cells and B cells that play a role as immunity against worm eggs and digestive tract protozoa (Ciriaco et al., 2003).

**Table 2.** Chi-Square Test Results Related to AgeDifferences in Layer Phase Laying Hens on WormEggs Prevalence

Age (weeks)	Positive	<b>Total Sample</b>
20-50	35	48
>50	42	48

Chi-Square test results showed no significant difference (P>0.05)
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**Table 3.** Chi-Square Test Results Related to AgeDifferences in Layer Phase Laying Hens onProtozoa Prevalence

Age (weeks)	Positive	Total Sample
20-50	25	48
>50	26	48

Chi-Square test results showed no significant difference (P>0.05)

Comparison of the age of layer phase between adult and old chickens showed a significant effect on the degree of infection of digestive tract worm eggs (P<0.05). The mean value of EPG in old chickens was higher than that of adult chickens, which can be observed in Table 4. Based on the results of the pre-research survey, farmers rarely conduct regular fecal examinations for early detection of digestive tract helminth egg infection. Laying hens infected with helminthiasis and no antihelmintic treatment will cause the development of worms in the host body to continue as the laying hen age so that the EPG value will be higher in older chickens (Tarbiat *et al.*, 2022).

**Table 4.** Mann-Whitney Test Results Related to AgeDifferences in Layer Phase Laying Hens on theDegree of Worm Eggs Infection

Age (weeks)	EPG
	Mean ± SD
20-50	$133.33^{a} \pm 118.65$
>50	$373.96^{b} \pm 450.41$

Different superscripts in the same column indicate significant differences (P<0.05)

Comparison of layer phase age between adult and old hens showed no significant effect on the degree of gastrointestinal tract protozoan infection (P>0.05). Adult and old layer hens have mean OPG that is not much different which can be observed in Table 5. These results can be caused by feed factors. Farmers sometimes give herbal products such as turmeric powder to their livestock. Turmeric powder is usually mixed into the feed as an additional feed. Herbal products work by stimulating the immune system of laying hens so that it can indirectly inhibit the development of *Eimeria* sp. in the host body (Martins *et al.*, 2022).

**Table 5.** Mann-Whitney Test Results Related to AgeDifferences in Layer Phase Laying Hens on theDegree of Protozoan Infection.

Age (weeks)	OPG
	Mean ± SD
20-50	$233.33 \pm 767.44$
>50	$296.87 \pm 600.92$
Monn Whitney, test manult	a showed no significant difference

Mann-Whitney test results showed no significant difference  $(P{>}0.05)$ 

## Conclusion

The identification results of endoparasites in the digestive tract of layer phase laying hens in Suruhwadang Village, Kademangan Sub-District, Blitar District were Ascaridia galli (68.75%), Heterakis gallinarum (53.12%), Strongyloides avium (5.21%), Trichostrongylus tenuis (6.25%), Eimeria maxima (51.04%), and Eimeria acervulina (3.12%). The highest mean infection degree of worm eggs and digestive tract protozoa was found in old laying hens.

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