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Diversity and prevalence of endoparasites in domestic chickens across an elevation gradient

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Abstract. *Setyowati EA, Santoso S, Rokhmani, Rochmatino. 2022. Diversity and prevalence of endoparasites in domestic chickens across an elevation gradient. Biodiversitas 23: 3936-3942.* The domestic chicken farm is negatively affected by endoparasites, which decrease immunity, egg production, and body weight. Although these effects are well understood in large-scale chicken farms, here we aimed to understand endoparasite diversity and prevalence in the context of local-scale domestic chicken farms conducted in Central Java, Indonesia, according to elevation. Chickens were sampled from three villages each in two regions; lowland in Banyumas District and highland in Purbalingga. The result showed that the diversity of endoparasite is very low (H' index: 1.3065-1.3773) and we detected only four endoparasite species (*Ascaridia galli, Trichuris trichiura, Heterakis gallinarum* (Nematoda), and *Raillietina* sp. (Cestoda) among a sample of 300 chickens. Endoparasite infection was significantly more prevalent in the lowland villages (70%) than in highland (48%,) ($p < 0.05$). Among endoparasites found, *A. galli* was the most prevalent species among all samples (50.0±0.0% of infections in the lowlands and 23.7±1.15% in the highlands) the second is *H. gallinarum* accounted for 38.3±7.64% of all infections in the highlands and 15.3±0.58% in the lowlands. *Raillietina* sp. accounted for 35.0±5.0% and 7.3±0.58% of all infections, and *T. trichiura* accounted for 25.0±5.0 % and 16.0±1.0%. The rate of endoparasite infection within the study area is currently moderate and mainly affected by methods of farm, our findings can serve as a baseline for controlling infection in domestic chickens.

Keywords: *Ascaridia galli*, Cestoda, *Heterakis gallinarum*, Nematoda, *Raillietina, Trichuris trichiura*

INTRODUCTION

Endoparasite infection in domestic chickens is a global issue impacting poultry productivity and native chicken species (Permin 2020; Shifaw et al. 2021). For example, Slimane (2016) documented endoparasites in various farm conditions and agricultural zones in Tunisia. Effects of nematode infections include reduced health, vigor, and production performance due to lower feed conversion ratios and growth rates, and/or weight loss, reduced egg production and quality, intestinal damage and, in severe cases, death (Mohammed et al. 2019). Aside from these direct effects, which largely stem from gastrointestinal damage, indirect effects like increased susceptibility to secondary infections and a decreased immune response can also negatively affect domestic chickens (Jaiswal et al. 2020). Tsegaye and Miretie (2021) showed that endoparasite infection results in immunosuppression, especially in response to vaccines against several poultry diseases. Of all intestinal worms, the large roundworm (*Ascaridia galli*) may inflict the most damage, with young chicks being more severely affected.

In Banyumas and Purbalingga Districts in Java, Indonesia, domestic chickens, i.e., those reared in small groups by individuals rather than in large-scale broiler chicken operations, populations exceed 1 million and 800,000, respectively, representing 10% and 20% of all broilers the two districts. The rearing of domestic chickens is an integral part of rural life in Java, in both highland and lowland areas, and allows families to improve their financial situation. Furthermore, domestic chickens are an important source of animal protein for rural populations (Zalizar et al. 2021). These chickens are typically raised using a free-range system, in which they scavenge around household compounds and feed on earthworms, insects, agricultural harvest residue, and human and animal waste. This free-range system influences the prevalence and severity of parasite attacks including ectoparasites (Riwidiharso et al. 2020) and endo- and intestinal parasites (Zalizar et al. 2021). Endoparasites are transmitted when chickens ingest parasite eggs directly in feces, or via food and water contaminated by feces, or by consuming grasshoppers or earthworms that carry parasites (Javaregowda et al. 2016).

The clinical signs of endoparasite infection are often not apparent, but infection may manifest as poor growth, decreased egg production, or death. In large-scale chicken farms, endoparasite outbreaks can cause substantial losses, but for traditional rural farmers, who often maintain <10 chickens, endoparasite infections often go unnoticed. Many studies on the prevalence of endoparasites in local chickens have been carried out by comparing various aspects. Bhat et al. (2014) compared chicken farms in humid areas with subtropical areas in India, compared local chickens slaughtered in Nigeria (Uhuo et al. 2013) and in Kenya (Junaidu et al. 2014), age and model rearing (Tsegaye and Mieritie 2021), local chicken that was scavenging with laying chicken in cages (Hariani and Simanjuntak 2021), between sexes (Mohammed et al. 2019), between agro-ecological zone (Slimane 2016), between seasons (Kumari and Bhagari 2018; Saraiva et al. 2020), different locations (Idika et al. 2016) and Win et al. (2020) between villages and town, and Van et al. (2019) who compared small-scale commercial flocks in the Mekong Delta Region of Vietnam. All the results of this study indicate that endoparasites infect domestic chickens with different prevalence rates. This difference in prevalence rates depends on the cage, environmental factors, age, sex and location. However, no research has been found that compares the prevalence of endoparasites in local chickens based on altitude. Research that approaches the study of altitude is the result of research by Slimane (2016), which compares chicken farms between agro-ecological zones. In Central Java, most of the topography is in the form of lowlands to highlands, where there are many local chicken farms. Therefore, this study aims to determine the diversity of endoparasite species and their prevalence in local chickens at different altitudes, the results of this study are expected to be used to determine endoparasite management policies, especially in traditional chicken farms with small-scale chickens.

MATERIALS AND METHODS

Study area

This study was conducted in lowland plains in Banyumas District and highland plains in Purbalingga District, Java, Indonesia. We sampled domestic chickens from three villages in each plain. Villages were selected based on elevation and the number of local chicken breeders. In Banyumas, we sampled from the villages of Kutasari (175m in elevation), Kedungwuluh (75m) and Kedungwringin (60m). In Purbalingga, we sampled from the villages of Serang (1124m), Kutabawa (1287m), and Ciwarak (1438m) (Figure 1, Table 1).

Endoparasite sampling and quantification

We sampled a total of 300 chickens; 50 chickens were sampled per village (25 females and 25 males in each sample). All chickens were approximately 8 months of age at the time of sampling, based on information obtained from their owners. Chicken feces were sampled directly from the cloaca using a sterile spatula and placed in a clean sample bottle. Each bottle was filled with 70% ethanol and placed on ice. Samples were then transported to the Entomology and Parasitology Laboratory of the Faculty of Biology at Jenderal Soedirman University, Purwokerto, Java, Indonesia. Samples were stored in a refrigerator at 4°C before being processed using the fecal flotation method. This method uses a solution of sodium chloride (NaCl) as a flotation fluid to detect Ascaris and heterocyst eggs in the laboratory. Floated samples were placed on slides, left for 10-15 minutes and then observed using a monocular microscope. Nematode eggs were identified using keys and descriptions provided by Soulsby (1986).

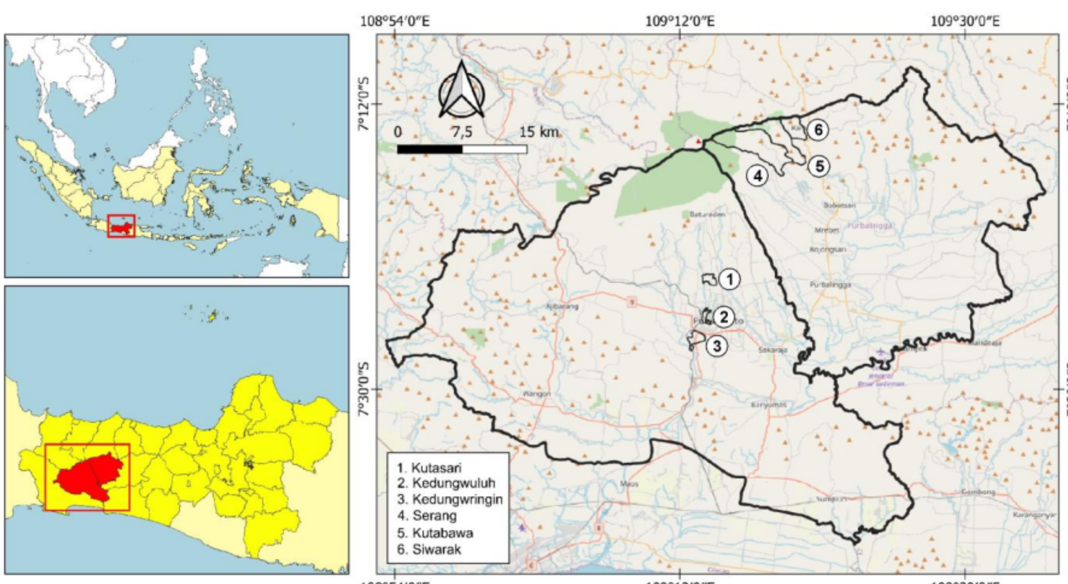


Figure 1. The six sampled villages in Banyumas and Purbalingga Districts, Central Java, Indonesia

Table 1. Average temperature, and average relative humidity for each of the six villages sampled from highland and lowland plains in Java, Indonesia

Parameter	Highland			Lowland		
	Serang	Kutabawa	Ciwarak	Kutasari	Kedungwuluh	Kedungringin
Temperature (^{0}C)	14-24	14-24	14-24	30-32	30-32	30-32
Humidity (%)	95-100	95-100	95-100	90-95	90-95	90-95

Data analysis

We assessed endoparasite diversity using multiple diversity indices, including the Shannon diversity index (H), Simpson diversity index (D), and evenness index (E), using the following equations:

$$H = \sum - (P_i * \ln P_i)$$

Where, P_i: the fraction of the entire population accounted for by a given species and ∑: the total number of species encountered;

$$D = (\sum n (n - 1)) / (N (N - 1))$$

Where, n: the number of individuals of a given species and N: the total number of individuals across all species; and

$$E = H'/Hmax$$

Where, H': a diversity index, Hmax: ln (S), and S: the total number of species.

Endoparasite prevalence was determined by dividing the number of infected samples by the total sample size, expressed as a percentage (by multiplying by 100). We then used ANOVA to determine differences in prevalence by elevation and sex, and between villages at the same elevation.

RESULTS AND DISCUSSION

Endoparasite species diversity in domestic chickens was low, we found four endoparasite species, *Ascaridia galli, Trichuris trichiura, Heterakis gallinarum* (Nematoda), and *Raillietina* sp. (Cestoda), among all samples (Table 2). There was no statistical difference in endoparasite diversity between the highland and lowland villages (H' index: 1.3065-1.3773). However, the total number of individuals varied between places, with the greatest number (n: 298) being found in Kedungwuluh at low elevation and the smallest number (n: 164) being found in Serang, at high elevation. The low species diversity of the research location may be explained by the incompatibility of environmental conditions for most of the endoparasite species. Based on the number of species, the result has no difference from the result of Zalizar et al. (2021), who found *A. galli, H. gallinarum, Raillietina* spp., and *Capillaria* spp. in domestic chickens from East Java. Tanuwijaya and Terbaldo (2021) found *A. galli, H. gallinarum, Capillaria caudinflata, Tetrameres americana,* and *Raillietina* sp. (Cestoda) in domestic chickens in Bali. In a study from Madura, East Java, Damayanti et al. (2019) found *Capillaria* sp.*, Raillietina* sp.*, Hymenolepis* sp., and *H. gallinarum*. Comparing with as other results showed that the number of species found this result has no difference from the general finding. In the work of Rufai and Jato (2017) and Ola-Fandusin et al. (2019), both reported endoparasite species and genera among domestic chicken from Nigeria that was also present in our study (*A. galli, H. gallinae, Syngamus trachea,* and *Capillaria annulata*). Shifaw et al. (2021) reported >30 helminth species from domestic chickens. Among these, *A. galli, H. gallinarum, Capillaria* spp., and *Raillietina* spp., were the most prevalent. Subedi et al. (2018) stated that most of the research results on endoparasites in local chickens always found *A. galli*, and *H. gallinarum. Ascaridia galli* and *H. gallinarum* are commonly reported parasitic and zoonotic nematodes of the chicken that lives in the small intestine. The high frequency of this parasite is likely due to its direct life cycle (Elele et al. 2021). Ingestion of water and food contaminated by infective eggs leads to the development of the egg into its larval stage when reaching the small intestine.

Based on the composition of the endoparasite species found in this study showed differences from the results of other studies conducted in Indonesia. Several other studies have found *Capillaria* sp. (Damayanti et al. 2019; Hariani and Simanjuntak 2021; Zalizar et al. 2021), *Hymenolepis* sp. (Damayanti et al. 2019), *Strongyloides* sp. (Kusuma et al. 2021), *Echinostoma revolutum*, *Raillietina echinobothrida*, *R. tetragona*, *Davainea proglottina*, *Amoebotaenia sphenoides*, and *Trichostrongylus tenuis* (Hariani and Simanjuntak 2021). This difference in species composition may be caused by differences in climate, especially rainfall, where our research location is in an area with high rainfall compared to other places. This is in line with the opinion of Uhuo et al. (2013) and Van et al. (2020), which state that endoparasite attack is highest in dry areas compared to wet areas.

Chicken kept in the backyard and free-range systems had a markedly higher pooled prevalence of helminth infection than those housed in cage production systems (Sherwin et al. 2013). However, in our study, only four helminth species were detected. This may be attributable to environmental conditions, where these four species may be the only ones able to reproduce in our study area. In general, the reported prevalence of helminth infections has decreased in some developing countries but has increased in poorly developed countries over time.

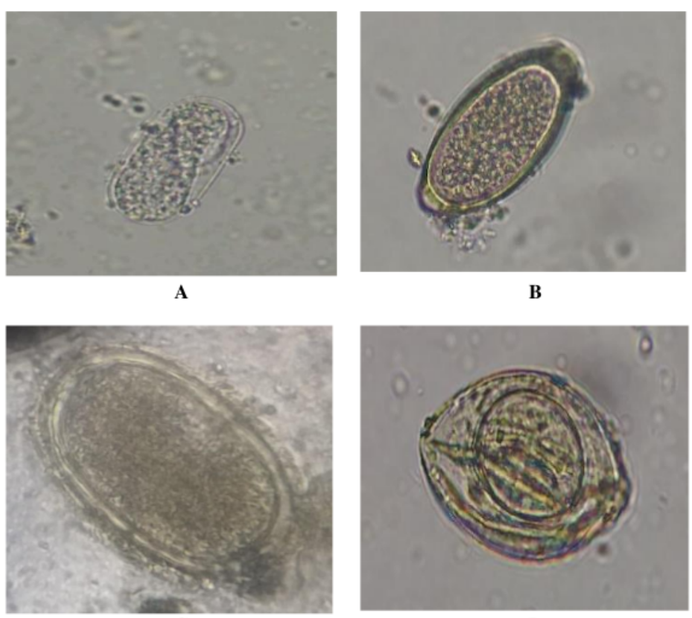
Within the two study districts, chickens are typically either free-scavenging or confined to the house or backyard. Farmers in Purbalingga District tend to use the latter method, confining their chickens to the home because of the general belief among villagers that free-scavenging chickens can damage crops. By contrast, farmers in Banyumas tend to allow their chickens to roam free. Free-roaming chickens are presumably more likely to encounter food sources that have been contaminated with chicken feces, thereby increasing their chances of contracting intestinal worms (Zalizar et al. 2021).

The number of endoparasite eggs observed in samples differed significantly (p<0.005) between the lowland and highland study areas; in total, there were 800 eggs in the lowland (298-237 per village) and only 514 in the highlands (179-161 per village). Kedungwuluh had the highest egg burden among lowland villages (n: 298), while Kutabawa had the highest burden among highland villages (n: 179). Differences in the number of individuals found between the highland and lowland villages were likely the product of environmental conditions, including the rearing methods described above and soil moisture. Domestic chickens in lowland areas forage in wider areas and thus may come into contact with a greater diversity of parasites than those in the highlands. Our results are consistent with Slimane (2016), who found that local chickens who foraged continuously in open, wild spaces were a greater chance of parasitic worm infection than those kept in cages (Imam et al. 2017).

Evennes (E) between sampling locations ranged between 0.9935 and 0.9424, this indicates that at the six sampling locations, both the highlands and lowlands have the same chance of attendance. This is as explained above about the diversity of endoparasite species which only 4 species were found. This finding is the same as the results of Zalizar et al. (2021). Damayanti et al. (2019) found the same 4 endoparasite species in Madura, more than the findings of Kusuma et al. (2021) in Jember, which only found 3 endoparasite species without finding *A. galli*. But is less than the results of Hariani and Simanjuntak (2021), who found 8 species of endoparasites with the greatest chance of *A. galli* in East Kalimantan.

Table 2. Diversity parameters of endoparasite species found in domestic chickens at six study sites

Diversity parameter	Highland			Lowland		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Species richness	4	4	4	4	4	4
No. individuals	164	179	171	265	298	237
Simpson (D)	0.2869	0.2935	0.2614	0.2544	0.2552	0.2580
Shannon (H)	1.3160	1.3065	1.3645	1.3773	1.3752	1.3692
Evenness (E)	0.9493	0.9424	0.9843	0.9935	0.9920	0.9876



A B C D

Figure 2. Eggs of endoparasites. A. *Ascaridia galli*; B. *Trichuris trichiura*; C. *Heterakis gallinarum*; D. *Raillietina* sp

Endoparasite prevalence

Domestic chickens in the lowland villages had a higher prevalence rate of endoparasites than those in the highland villages (70% and 48%, respectively, $p < 0.05$) (Table 3). These results are consistent with those of Rufai and Jato (2017), who reported a higher prevalence of endoparasites in lowland than highland sites. This is likely due to temperature and humidity differences between these regions in association with altitude (Ola-Fandusin et al. 2019; Shifaw et al. 2021). Soil moisture and temperature, which are driven by air temperature and humidity, affect the longevity of parasite eggs (Berhe et al. 2019; Win et al. 2020; García-Cuadrado et al. 2021,). Alam et al. (2014) also reported differences in endoparasite prevalence in domestic chickens among different ecological zones.

Among all endoparasite species observed in sampled domestic chickens, *A. galli* was the most prevalent, accounting for 50.0±0.0% of infections in the lowlands and 23.7±1.15% in the highlands. *H. gallinarum* accounted for 38.3±7.64% of all infections in the highlands and 15.3±0.58% in the lowlands. *Raillietina* sp. accounted for 35.0±5.0% and 7.3±0.58% of all infections, respectively, while *T. trichiura* accounted for 25.0±5.0% and 16.0±1.0%, respectively (Table 4). The high prevalence of *A. galli* was expected; it is the primary parasite of domestic chickens worldwide (Sharma et al. 2017). According to Wongrak et al. (2014), *A. galli* is one of the most common gastrointestinal parasites found in laying hens. The prevalence of this parasite, according to several studies, ranges from 22-84% of the total parasite load (Sherwin et al. 2014). The higher prevalence of *A. galli*, because of the direct life cycle and thus infection can spread among scavenging chickens as they are in constant contact with manure and soil (Wongrak et al. 2014), and also the eggs are resistant to the external environment (Tarbiat et al. 2015). After inoculation, the embryonated *A. galli* eggs hatch in the small intestine of the host. The released larvae can cause extensive damage and erosion of the intestinal mucosa as well as the proliferation of mucus-secreting cells. *A. galli* infection is often associated with decreased body condition, increased feed conversion ratio, and decreased overall health. The infection can also act to suppress the host's immune system, thereby increasing the severity of the concomitant disease. According to Sharma et al. (2017) and Wongrak et al. (2014), *A. galli* is the main endoparasite in local chickens in various places, with a prevalence between 22-84%. The prevalence level differs between locations mainly due to climatic factors, environment, and cultivation methods.

The second highest prevalence was *H. gallinarum* which was 38.3±7.64% this was due to the nature of the eggs of this worm which could survive in infective conditions in the soil in the long term, as well as the presence of paratenic hosts in earthworms, so this species of worm very easily eaten by wild chickens (Papini and Cacciutollo 2008).

Table 3. Occurrence and prevalence of endoparasites in domestic chickens, and associated p-values, by elevation, location, and sex. *indicates significance at $p < 0.05$

Source of variation	n	Infected	Not infected	Prevalence (%)	p-value
Elevation					**0.00***
Highland	150	72	78	48	a
Lowland	150	105	45	70	b
Village (Highland)					**0.934**
Serang	50	24	26	48	
Kutabawa	50	23	27	46	
Ciwarak	50	22	28	44	
Sex (Highland)					**0.074**
Cock	75	40	35	53	
Hen	75	33	42	44	
Village (Lowland)					**0.934**
Kutasari	50	33	17	66	
Kedungwuluh	50	34	16	68	
Kedungwringin	50	37	13	74	
Sex (Lowland)					**0.074**
Cock	75	53	22	70	
Hern	75	54	21	72	

Table 4. Endoparasite species prevalence in domestic chickens in two study regions. (*) indicates significant differences among rows

Species	Highland			Prevalence (%) Mean ± stdev	(*)	Lowland			Prevalence (%) Mean ± stdev	(*)
	Sampling location					Sampling location				
	1	2	3			1	2	2		
A. galli	25	23	23	23.7 ± 1.15	A	50	50	50	50 .0 ± 0.0	c
T. trichura	17	15	16	16.0 ± 1.0	B	20	30	25	25.0 ± 5.0	d
Raillietina sp.	7	8	7	7.3 ± 0.58	B	35	40	30	35.0 ± 5.0	d
H. gallinarum	16	15	15	15.3 ± 0.58	B	30	40	45	38.3 ± 7.64	d

In addition to the two species mentioned above, this study found the third highest prevalence of the species *Raillietina* sp, which was 35.0±5.0. This is due to the fact that *Raillietina* sp is an important Cestode in the life of local chickens, is cosmopolitan, widely distributed, and transmitted by ants, flies and ground beetles, so it is easily transmitted and is present in wild chicken farming models (Gamra et al. 2015).

The three species of endoparasites, namely 2 Nematodes (*Ascaridia galli* and *Heterakis gallinarum*) and 1 Cestoda (*Raillietina* sp.), are the main endoparasites with a high prevalence rate in local and laying hens, this is evident from the results of research in various places that have been conducted between others Bhat et al. (2014) in the North Indian Region prevalence of *A. galli* 19.6%, *H. gallinarum* 9.5% and *Raillietina sp*. 16.6%, In Karnatake India, the highest prevalence was *Raillietina sp* (77.6%) (Javaregowda et al. 2016). Shifaw et al. (2021) stated that the average prevalence of the three endoparasite species was *A. galli* (35.9%), *H. gallinarum* (28.5%) and *Raillietina sp* (19%). Even in Tunisia (Slimane 2016), *H. gallinarum* prevalence was found to be 100%, *A. galli* (53%). *Raillietina* sp. (33%). Meanwhile, data from research in Ethiopia (Berhe et al. 2019) showed the same results, namely the highest prevalence of *H. gallinarum* (72%) compared to *A. galli* of (68.85%).

The high level of prevalence of the three endoparasite species at the location of this research, in addition to the biological nature and presence of the three species is also caused by external factors, which include: the climatic conditions and ecological zones, the accumulation of infective stages of larvae or eggs in the environment, the presence of intermediate hosts, and the individual susceptibility of the final host. Temperature and humidity can be considered determinants for the occurrence and the level of helminth infection by influencing transmission through survival in the environment and developmental success of the infective stage (Sharma et al. 2017). In addition, other determining factors are the cultivation model, which is scavenging or in the cage, the quality of feed and the cleanliness of the cage. Because scavenging chickens will have a higher chance of contact with endoparasite worm eggs than caged chickens, the level of cleanliness of the cage, especially from chicken feces, will also determine the prevalence of endoparasites because the chances of contact are higher in dirty and unhygienic cages. The quality of feed will greatly determine the chicken's resistance to endoparasite attacks (Subedi et al. 2018).

Based on the results and previous discussion, it can be concluded that the diversity of endoparasite species in local chickens is very low, namely, only 4 species are found, and the prevalence of endoparasites in local chickens is higher in the lowlands than in the highlands, while between sampling locations at the same altitude there is no difference. The highest prevalence was in *A. galli*, followed by *H. gallinarum* and *Raillietina* sp and the lowest was in *T. trichiura* species.

The results of this study recommend traditional local chicken farmers limit the local chicken foraging area, clean the forage location and improve the quality of the feed to reduce the risk of being exposed to endoparasites.

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