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ENDANG ARIYANI SETYOWATI, SLAMET SANTOSO, ROKHMANI, ROCHMATINO

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Endang Ariyani Setyowati

Diversity and Prevalence of Endoparasites in Domestic Chickens Across an Elevation Gradient

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Abstract. Domestic aviculture is negatively affected by endoparasites, which decrease immunity, egg production, and body weight in domestic chickens. Although these effects are well understood in large-scale aviculture, here we aimed to understand endoparasite diversity and prevalence in the context of local-scale domestic chicken breeding 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. We detected four endoparasite species (*Ascaridia galli, Trichuris trichura, Heterakis gallinarum* (Nematoda), and *Raillietina sp* (Cestoda) among a sample of 300 chickens. Endoparasite infection was significantly more prevalent in the lowland villages and *A. galli* was the most prevalent species among all samples (prevalence rate of 50%). The rate of endoparasite infection within the study area is currently moderate and our findings can serve as a baseline for controlling infection in domestic chickens.

Keywords: *Ascaridia galli*, *Heterakis gallinarum*, Nematoda, *Trichuris trichura, Raillietina* sp., Cestoda

Running title: Endoparasites in domestic chickens in different elevations

INTRODUCTION

Endoparasite infection in domestic chickens is a global issue impacting poultry productivity and native chicken species (Shifaw et al., 2021, Permin, 2020). 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., 2021). 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 chicken, 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). 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 sub-tropical 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 were scavenging with laying chicken in cages (Hariani and Simanjuntak, 2021), between sexes (Mukaratirwa and Khumalo, 2010; Mohammed et la., 2019), between agro-ecological zone (Slimane et al., 2016) , between seasons (Kumari and Bhagari, 2018; Saraiva et al., 2020), different locations (Idika et al, 2016) and Win et al., (2019) between villages and town, and Van et al., (2019) who compared small-scale commercial flocks in the Mekong Delta Region of Vietnam. The results of these studies all show that endoparasites are infected with different prevalence levels and different endoparasite species compositions. 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. different. Here, we will quantify the prevalence of endoparasite infections in domestic chickens reared using free-range systems in rural areas across an elevational gradient.

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 (175 m in elevation), Kedungwuluh (75 m) and Kedungwringin (60 m). In Purbalingga, we sampled from the villages of Serang (1,124 m), Kutabawa (1,287 m), and Ciwarak (1,438 m) (Fig. 1, Table 1).

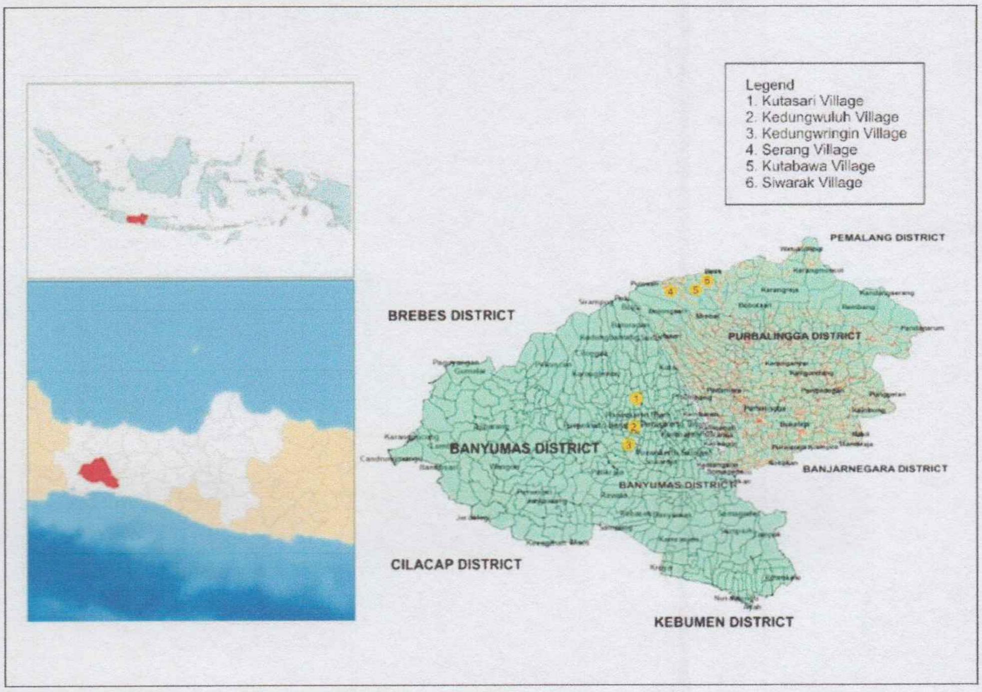

Figure 1. The six sampled villages in Banyumas and Purbalingga Districts, Java, Indonesia.

Table 1. Elevation, domestic chicken population, 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

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).

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 follow equations:

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

Where P_i = the fraction of the entire population accounted for by a given species and $\sum$ = 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

RESULTS AND DISCUSSION

Endoparasite species diversity in domestic chickens was low, we found four endoparasite species, *Ascaridia galli, Trichuris trichura, Heterakis gallinarum* (Nematoda), and *Reiletina* sp. (Cestoda) among all samples (Table 2). There was no 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 with 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 others result showed that the number of species found at this result has no difference with general finding. In the work of Rufai and Jato (2017) and Fandusin et al. (2019), who both reported endoparasite species and genera among domestic chicken from Nigeria that were also present in our study (*A. galli, H. gallinae, Syngamus trachea, and Capillaria annulate*). Shifaw (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*.

A.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. (Ybanez et al., 2018).

Based on the composition of the endoparasite species found in this study, showed differences with the results of other studies conducted in Indonesia. Several other studies have found *Capillaria sp*, (Zalizar et al., 2021; Damayanti et al., 2019; Hariani and Simanjuntak, 2021), *Hymenolepis sp*. (Damayanti et al., 2019), *Strongyloides sp*. (Kusuma et al., 2019) , *Echinostoma revolutum*, *Raelleitina echinobothrida*, *R. tetragona*, *Davinea proglotina*, *Amoebotaenia sphenoides*, and *Trichostongylus 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 states that endoparasite attack is highest in dry areas compared to wet areas.

Chicken kept in 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 agricultural 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).

Table 1. 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

The number of endoparasite eggs observed in samples differed significantly 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 at 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 in 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., (2022). Damayanti et al., (2019) who 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.

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, $p < 0.05$). Prevalence did not vary significantly among villages in the highlands or lowlands, or between sexes (Table 2). 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 (Shifaw et al., 2021, Ola-Fandusin et al., (2019). 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.

Table 2. 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
1. Elevation					0.00*
Highland	150	72	78	48	a
Lowland	150	105	45	70	b
2. Village (Highland)					0.934
Serang	50	24	26	48	
Kutabawa	50	23	27	46	
Ciwarak	50	22	28	44	
3. Sex (Highland)					0.074
Cock	75	40	35	53	
Hen	75	33	42	44	
4. Village (Lowland)					0.934
Kutasari	50	33	17	66	
Kedungwuluh	50	34	16	68	
Kedungwringin	50	37	13	74	
5. Sex (Lowland)					0.074
Cock	75	53	22	70	
Hern	75	54	21	72	

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. trichura* 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 chicken as they are in constant contact with manure and soil (Wongrak et al., 2014) and also the eggs are resistant to the external environment (Tarbiet 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 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 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 had the ability to survive and 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. Endoparasite species prevalence in domestic chickens in two study regions. (*) indicates significant differences among rows.

Species	Highland			Prevalence (%)	(*)	Lowland			Prevalence (%)	(*)
	Sampling location			Mean ± stdev		Sampling location			Mean ± stdev	
	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, 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%) (Javaredowdha 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 et al., 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 as 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 (Yousaf et al. al., 2019). The quality of feed will greatly determine the chicken's resistance to endoparasite attacks (Subedi et al., 2020).

Conclussion

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, 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. trichura* species.

The results of this study recommend traditional local chicken farmers to 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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REFERENCE

Alam N , Mostofa M , Khan MHNA , Alim MA , a. K. M. A. Rahman AKMA, Trisha A. 2014. Prevalence of gastrointestinal helminth infections in indigenous chickens of selected areas of Barisal district, Bangladesh Bangl. J. Vet. Med. 12 (2): 135-139

Berhe M , Mekibib B , Bsrat A , Atsbaha G. 2019. Gastrointestinal Helminth Parasites of Chicken under Different Management System in Mekelle Town, Tigray Region, Ethiopia. Journal of Veterinary Medicine Volume 2019, Article ID 1307582, 7 pages https://doi.org/10.1155/2019/1307582

Damayanti EA, Hastutiek P, Estoepangestie ATS, Retno NR , Kusnoto, Suprihati E . 2019. The Prevalence and Infection's Degree of Gastrointestinal Worm of Local Chicken (*Gallus Domesticus*) in Kramat Village, District of Bangkalan, Madura, East Java Indonesia. Journal of Parasite Science 3 (1). https://doi.org/10.20473/jops.v3il.16436

Elele K, Amawulu E, Eniyekedidei KP. 2021. Parasites Status of Village Chickens (*Gallus gallus domesticus*) in Selected Communities in Yenagoa Local Government Area of Bayelsa State, Nigeria. Open Journal of Applied Sciences, 11, 230-238. https://doi.org/10.4236/ojapps.2021.112016

Gamraa OW, Antiab RE, Falohunc OO. 2015. Intestinal cestodes of poultry *Raillietina echinobothrida* and *Choanotaenia Infundibulum* infection in a commercial Japanese quail (*Coturnix coturnix japonica*) farm in Apomu, Osun State, Nigeria. Scientific Journal of Zoology. 4(4) 20-25 https://doi.org/10.14196/sjz.v4i4.1860

García-Cuadrado MN, F. J. Martínez-Moreno FJ, Leva RZ, García IA. 2021. Helminth communities in the alimentary tract of free raised chickens on rainfed and irrigated agrosystems from southwest Spain, Italian Journal of Animal Science, 20:1, 1689-1694. https://doi.org/10.1080/1828051X.2021.198044

Hariani N, Simanjuntak I. 2021. Prevalence and Intensity of Parasitic Worms on Free-Range Chickens and Egg-Laying Chickens in Muara Badak sub-District, Kutai Kartanegara Jurnal ILMU DASAR, 22 (1) 1-8

Idika K, Obi CF, Ezeh IO, Iheagwam CN, Njoku IN, Nwosu CO. 2016. Gastrointestinal helminth parasites of local chickens from selected communities in Nsukka region of south eastern Nigeria. J Parasit Dis. 40(4):1376–1380. https://doi.org/10.1007/s12639-015-0694-9

Imam TS , Dambo R, Sa'id MA, Suleiman K. 2017. Detection of Gastro-Intestinal Helminthes in Local Chicken (*Gallus gallus*) Sold at Sharada Market, Kano Metropolis, Nigeria . Journal Dryland Agriculture. 3(1). 19 – 27

Jaiswal K, Mishra S, Bee A. 2020. Prevalence of Gastrointestinal Helminth Parasites in *Gallus gallus domesticus* in Lucknow, U. P, India. Advances in Zoology and Botany,8(5), 422-430. https://doi.org/10.13189/azb.2020.080506

Javaregowda AK, Rani BK, Revanna SP, Udupa G .2016. Prevalence of gastro-intestinal parasites of backyard chickens *(Gallus domesticus)* in and around Shimoga. J Parasit Dis 40(3):986–990. https://doi.org/10.1007/s12639-0140620-6

Junaidu HI, Luka SA, Mijinyawa A. 2014. Prevalence of Gastrointestinal Helminth Parasites of the Domestic fowl (*Gallus-gallus domesticus*) slaughtered in Giwa Market, Giwa Local Government, Area, Kaduna State, Nigeria Journal of Natural Sciences Research. 4. (19) 120-128

Kusuma SB, Nusantoro S,Muhamad N, Awaludin A, Hasanah N, Adhytama M.2021. Identification of helminth parasite diversity on laying chicken in Jember (East Java-Indonesia). IOP.Conf.Series: Earth and Environmental Science. 672. 012045. https://doi.org/10.1088/1755-1315/i/012045

Mohammed BR, Ojo AO, Opara MN, Jegede OC, Rowland I.S. Agbede RIS. 2019. Haemo and endoparasites of indigenous chickens reared in Gwagwalada Area Council, Abuja, Nigeria. Annals of Parasitology. 65(3), 293-296 https://doi.org/10.17420/ap6503.213

Mwale M, Masika PJ. 2011. Point prevalence study of gastro-intestinal parasites in village chickens of Centane district, South Africa African Journal of Agricultural Research. 6 (9). 2033-2038, https://doi.org/10.5897/AJAR09.495

Ngongeh LA, Chiejina SN, Lawal AI. 2014. Prevalence of gastrointestinal helminth infections in slaughtered chickens reared in the Nsukka area of Enugu State, Nigeria. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS). 7, (11) 51-54

Nguyen TBV, Nguyen VC, Nguyen TP, Nguyen TH, Kiet BT, Nguyen VH, Hien VB, Thwaites G, Carrique-Mas JJ, Ribas A. 2020. Characterisation of gastrointestinal helminths and their impact in commercial small-scale chicken flocks in the Mekong Delta of Vietnam Tropical Animal Health and Production 52:53–62. https://doi.org/10.1007/s11250-019-01982-3

Ola-Fadunsin SD, Uwabujo PI, Sanda IM, Ganiyu IA, Hussain K, Rabiu M, Elelu N, Alayande MO. 2019. Gastrointestinal helminths of intensively managed poultry in Kwara Central, Kwara State, Nigeria: Its diversity, prevalence, intensity, and risk factors, Veterinary World, 12(3): 389-396.

Permin A. 2020. Impact of Helminth Infections on Production of Chickens. Biomedical. Journal of Scientific & Technical Research. 29.(3). https://doi.org/10.26717/BJSTR.2020.29.004819

Papini R, Cacciuttolo E. 2008. Observations on the occurrence of *Heterakis gallinarum* in laying hens kept on soil. Ital.J.Anim.Sci. vol. 7, 487-493.

Riwidiharso E, Darsono, Setyowati EA, Pratiknyo H, Sudiana E, Santoso S, Yani E, Widhiono I. 2020.Prevalence and diversity of ectoparasites in scavenging chickens *(Gallus domesticus)* and their association to body weight. Biodiversitas. 21(7) 3163-3169 https://doi.org/10.13057/biodiv/d210738

Rufai MA, Jato AO. 2017. Assessing The Prevalence Of Gastrointestinal Tract Parasites Of Poultry And Their Environmental Risk Factors In Poultry In Iwo, Osun State Nigeria. Ife Journal of Science. 19 (1). 2-13. https://dx.doi.org/10.4314/ijs.v19i1.2

Saraiva DJ, Campina ACC, Gonçalves FCS, Melo-Viegas D, Santos ACG, Nogueira RMS, Costa AP, Gastrointestinal Parasites in Free-Range Chicken Raised under Extensive System from the Northeast of Brazil . Brazilian Journal of Poultry Science Revista Brasileira de Ciência Avícola http://dx.doi.org/10.1590/1806-9061-2020-1337.

Sharma N, Hunt PW, Hine BC, Swick RA, Sharma NK, Ruhnke I .2017. *Ascaridia galli* challenge model in laying hens. J. Adv. Parasitol. 4(3): 41-46. DOI | http://dx.doi.org/10.17582/journal.jap/2017/4.3.41.46

Shifaw A, Feyera T, Stephen W,Brown W, Sharpe B, Elliott T, Ruhnke I .2021. Global and regional prevalence of helminth infection in chickens over time: a systematic review and meta-analysis. Poultry Science 100:101082 https://doi.org/10.1016/j.psj.2021.101082

Sherwin C M, Nasr M A, Gale E, Petek M, Stafford K, Turp M, Coles G C. 2014. Prevalence of nematode infection and faecal egg counts in free-range laying hens: relations to housing and husbandry. Br. Poult. Sci. 54(1):12-23. https://doi.org/10.1080/00071668.2012.757577

Slimane BB. 2016. Prevalence of the gastro-intestinal parasites of domestic chicken Gallus domesticus Linnaeus, 1758 in Tunisia according to the agro-ecological zones. J Parasit Dis 40(3):774–778. https://doi.org/10.1007/s12639-014-0577-5

Subedi JR, Mujahid T, Cheetri B. 2018. Prevalence of intestinal helminth parasites of chicken *(Gallus gallus domesticus* Linnaeus, 1758) in Lalitpur district, Nepal. Tribhuvan University Journal, vol. 32, no. 2,

Tanuwijaya PA, Febraldo D. Parasite Infections In Poultry Environments (Case Report On Gallus Domesticus Endoparasite) Journal of Environmental Science and Sustainable Development 4 (1). 97-136. https://doi.org/10.7454/jessd.v4i1.1083

Tarbiat B, Jansson DS, Höglund J. 2015. Environmental tolerance of free-livingstages of the poultry roundworm Ascaridia galli. Vet. Parasitol. 209: 101–107. https://doi.org/10.1016/j.vetpar.2015.01.024

Tsegaye AA, Miretie AA. 2021. Chicken Ascariasis and Heterakiasis: Prevalence and Associated Risk Factors, in Gondar City, Northwest Ethiopia. Veterinary Medicine: Research and Reports 12. 217–223. https://doi.org/10.2147/VMRR.S323284

Uhuo AC, Okafor FC, Odikamnoro OO, Onwe CS, Abarike MC, Elom JN. 2013. Common gastrointestinal parasites of local chicken (gallus domesticus) slaughtered in some selected eatery centres in Abakaliki, Ebonyi State: Implication for meat quality. International Journal of Development and Sustainability 2 (2), 1416-1422.

Vieira FEG, Yamamura MH, Freire FR, Headley SA. 2015. The effects of managerial systems on helminth infection in freerange chickens from northern Paraná, Semina: Ciências Agrárias, Londrina 36 (6) 4311-4322. http://dx.doi.org/10.5433/1679-0359.2015v36n6Supl2p4311

Win SY, Htun LL, Hmoon MM, Chel HM, Thaw YN, Soe NC, Oo HL, Bawm S. 2020. Occurrence of gastrointestinal parasites in free ranging village chickens from four townships of Myanmar. Veterinary Sciences: Research and Reviews, 6(1): 1-6. http://dx.doi.org/10.17582/journal.vsrr/2020/6.1.1.6

Wongrak K, Das G, Moors E, Sohnrey B, Gauly M. 2014. Establishment of gastro-intestinal helminth infections in free-range chickens: a longitudinal on farm study. Berlin Munich. Tierarztl. Wochenschr. 127: 314–321

Yassir Dakheel Kremsh Alasadiy , Arshad Naji Alhasnawi1 , Farhan AlaAllah Eabaid1 The Prevalence of Parasitic Infection in Domestic Chicken: A Review Medico-legal Update, January-March 2020, Vol.20, No. 1 https://doi.org/10.37506/v20/i1/2020/mlu/194434

Yousaf A, Tabasam MS, Memon A, Rajput N, Shahnawaz R, Rajpar S, Jamil T, Mushtaq M. 2019. Prevalence of *Ascaridia galli* in different broiler poultry farms of potohar region of rawalpindi pakistan. Journal of Dairy, Veterinary & Animal Research.Volume 8 Issue 1 - 2019

Yousfi F, Senouci K , Medjoual I, Djellil H, Slimane TH. 2013. Gastrointestinal helminths in the local chicken Gallus gallus domesticus (Linnaeus, 1758) in traditional breeding of North Western Algeria Biodiversity Journal. 4 (1): 229-234

Zalizar L, Winaya A, Malik A, Widodo W, Suyatno, Anggraini AD. 2021 Species identification and prevalence of gastrointestinal helminths in Indonesian native chickens, and its impact on egg production. Biodiversitas. 22(10) 4363-4369 https://doi.org/10.13057/biodiv/d221029

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Dari: **Ayu Astuti** <smujo.id@gmail.com>
Date: Jum, 15 Jul 2022 16:25
Subject: [biodiv] Editor Decision
To: setyowati endang ariyani <endang.setyowati@unsoed.ac.id>, slamet santoso <slamet.santoso@unsoed.ac.id>, rokhmani rokhmani <rokhmani@unsoed.ac.id>, rochmatino rochmatino <rochmatino@unsoed.ac.id>

setyowati endang ariyani, slamet santoso, rokhmani rokhmani, rochmatino rochmatino:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Diversity and Prevalence of Endoparasites in Domestic Chickens Across an Elevation Gradient".

Our decision is: Revisions Required

Reviewer F:

Strengths
1. The study objective is clear and concise.

2. The study showed a significant prevalence of endoparasite in the lowlands than the highlands.

Areas to Improve

1. The et al. in the in-text citation should be italicized.

2. Some major compound sentences need rephrasing.

3. Table numbering (Line 142 and Line 170) should be checked.

4. Line 166, Role of soil moisture and temperature needs further explanations.

Recommendation: Revisions Required

Reviewer H:

Generally, the study was conducted to find out the diversity and prevalence of endoparasites in domestic chickens across an elevation gradient in Central Java, Indonesia. The study is relevant as it was conducted to address a major issue facing local chicken farmers. Though this manuscript is relevant, it is currently having some major issues which need to be addressed before it can be accepted.

Dari: **Ayu Astuti** <smujo.id@gmail.com>
Date: Sel, 26 Jul 2022 23:30
Subject: [biodiv] Editor Decision
To: ENDANG ARIYANI SETYOWATI <endang.setyowati@unsoed.ac.id>, SLAMET SANTOSO <slamet.santoso@unsoed.ac.id>, ROKHMANI <rokhmani@unsoed.ac.id>, ROCHMATINO <rochmatino@unsoed.ac.id>

ENDANG ARIYANI SETYOWATI, SLAMET SANTOSO, ROKHMANI, ROCHMATINO:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Diversity and prevalence of endoparasites in domestic chickens across an elevation gradient".

Our decision is to: Accept Submission

Biodiversitas Journal of Biological Diversity

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Dari: **Smujo Editors** <smujo.id@gmail.com>
Date: Sab, 13 Agu 2022 06:50
Subject: [biodiv] Editor Decision
To: ENDANG ARIYANI SETYOWATI <endang.setyowati@unsoed.ac.id>, SLAMET SANTOSO <slamet.santoso@unsoed.ac.id>, ROKHMANI <rokhmani@unsoed.ac.id>, ROCHMATINO <rochmatino@unsoed.ac.id>

ENDANG ARIYANI SETYOWATI, SLAMET SANTOSO, ROKHMANI, ROCHMATINO:

The editing of your submission, "Diversity and prevalence of endoparasites in domestic chickens across an elevation gradient," is complete. We are now sending it to production.

Submission URL: https://smujo.id/biodiv/authorDashboard/submission/11488

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

Abstract. Domestic chicken farm is negatively affected by endoparasites, which decrease immunity, egg production, and body weight in domestic chickens. Although these effects are well understood in large-scale chicken farm, here we aimed to understand endoparasite diversity and prevalence in the context of local-scale domestic chicken farm 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 endoaparsite is very low (H' index: 1.3065–1.3773) and we detected only four endoparasite species (*Ascaridia galli, Trichuris trichura, 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 endoparasite 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. trichura* 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, Heterakis gallinarum, Nematoda, Trichuris trichura, Raillietina sp., Cestoda

Running title: Endoparasites in domestic chickens in different elevations

INTRODUCTION

Endoparasite infection in domestic chickens is a global issue impacting poultry productivity and native chicken species (Shifaw et al., 2021, Permin, 2020). 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., 2021). 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 chicken, 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. differentMany 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 sub-tropical areas in India, compared local chickens slaughtered in

Comment [U1]: I suggest you delete the domestic chicken at the end of the sentence since it has already been mentioned at the start.

Comment [U2]: Should be plural "farms"

Comment [U3]: This should be plural "farms"

Comment [U4]: Try to rephrase this sentence; "Although these impacts in large-scale chicken farms are well established, the aim of this study was to comprehend endoparasite diversity and prevalence in small-scale domestic chicken farms in Central Java, Indonesia, according to elevation.

Comment [U5]: please check the spelling of "endoparasite"

Comment [U6]: Could you please state the sampling procedure in the abstract.

Comment [U7]: This should be plural "endoparasites"

Comment [U8]: Methods of farming rather than farm

Comment [U9]: Please scientific names should be in italics

Comment [U10]: Should be in italic

Comment [U11]: I suggest you state the outcome of Slimane's work?

Comment [U12]: Could you please rephrase and link these two sentences into one?

Comment [U13]: Please provide a reference to this statement.

Comment [U14]: Please rephrase this statement.

Comment [U15]: Please italicize the et al. in your work.

Comment [U16]: The "different" should be deleted.

Comment [U17]: Please provide a few references to back this claim.

Nigeria (Uhuo et al, 2013) and in Kenya (Junaidu et al., 2014), age and model rearing (Tsegaye and Mieritie, 2021), local chicken that were scavenging with laying chicken in cages (Hariani and Simanjuntak, 2021), between sexes (Mukaratirwa and Khumalo, 2010; Mohammed et la., 2019), between agro-ecological zone (Slimane et al., 2016) , between seasons (Kumari and Bhagari, 2018; Saraiva et al., 2020), different locations (Idika et al, 2016) and Win et al., (2019) between villages and town, and Van et al., (2019) who compared small-scale commercial flocks in the Mekong Delta Region of Vietnam. The results of these studies all show that endoparasites are infected with different prevalence levels and different endoparasite species processes depending on cage, environment 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 et al., (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. However, research that compares the prevalence of endoparasites in local chickens based on altitudes has not been reported. 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~~.

Comment [U18]: Could you please limit the comparisons to two or three and state the results obtained?

Comment [U19]: Could you please rephrase this sentence?

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 (175 m in elevation), Kedungwuluh (75 m) and Kedungwringin (60 m). In Purbalingga, we sampled from the villages of Serang (1,124 m), Kutabawa (1,287 m), and Ciwarak (1,438 m) (Fig. 1, Table 1).

Comment [U20]: Could you please state the sampling technique employed?

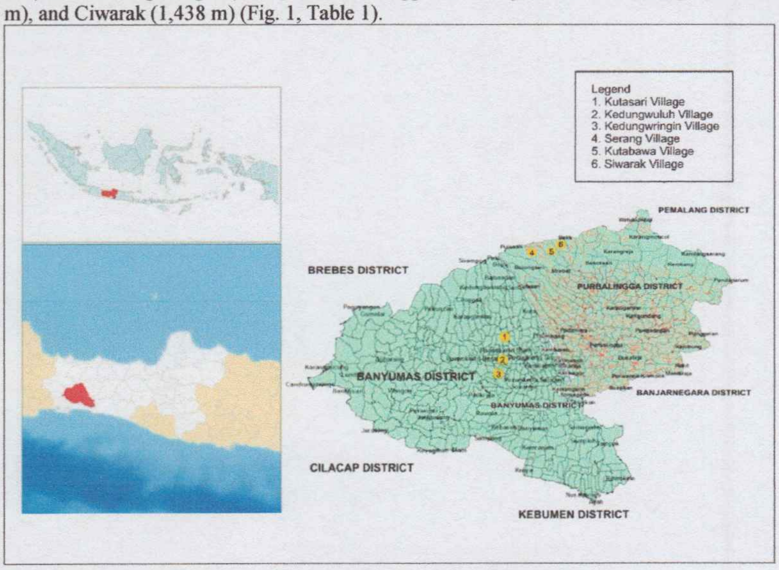

Figure 1. The six sampled villages in Banyumas and Purbalingga Districts, Java, Indonesia.

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

Comment [U21]: I think your table only captured the average temperature and relative humidity. Could you provide the data for elevation and domestic chicken pollution in the six villages?

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

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

Comment [U22]: Please provide the name of the sample bottle used.

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).

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 follow equations:

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

Where P_i = the fraction of the entire population accounted for by a given species and $\sum$ = 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 trichura, Heterakis gallinarum* (Nematoda), and *Reiletina* sp. (Cestoda) among all samples (Table 2). There was no 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 with 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 others result showed that the number of species found at this result has no difference with general finding. In the work of Rufai and Jato (2017) and Fandusin et al. (2019), who both reported endoparasite species and genera among domestic chicken from Nigeria that were also present in our study (*A. galli, H. gallinae, Syngamus trachea, and Capillaria annulate*). Shifaw (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*. *A.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. (Ybanez et al., 2018).

Based on the composition of the endoparasite species found in this study, showed differences with the results of other studies conducted in Indonesia. Several other studies have found *Capillaria sp*, (Zalizar et al., 2021; Damayanti et al., 2019; Hariani and Simanjuntak, 2021), *Hymenolepis sp*. (Damayanti et al., 2019), *Strongyloides sp*. (Kusuma et al., 2019) , *Echinostoma revolutum*, *Raelleitina echinobothrida*, *R. tetragona*, *Davinea proglotina*, *Amoebotaenia sphenoides*, and *Trichostongylus 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 states that endoparasite attack is highest in dry areas compared to wet areas.

Chicken kept in 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

Comment [U23]: Please check the Table Numbering.

Comment [U24]: This sentence is not clear. Please rephrase it.

Comment [U25]: Please provide the relation between this reference and your study

Comment [U26]: Please provide further explanations on the relationship between direct life cycle and high frequency.

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 agricultural 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).

Table 1. 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

The number of endoparasite eggs observed in samples differed significantly 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 at greater chance of parasitic worm infection than those kept in cages (Imam et al., 2017)

Comment [U27]: Please explain further on how soil moisture could account for the difference in the number of parasites between low lands and highland.

Evennes (E) between sampling locations ranged between 0.9935 and 0.9424, this indicates that at the six sampling locations, both in 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., (2022). Damayanti et al., (2019) who 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.

Comment [U28]: Please state what could have accounted for the species disparities.

Comment [U29R28]: Please rephrase.

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, $p < 0.05$). Prevalence did not vary significantly among villages in the highlands or lowlands, or between sexes (Table 2). 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 (Shifaw et al., 2021, Ola-Fandusin et al., (2019). 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.

Comment [U30]: Please state how Alam et al. study relates to your discussion.

Table 2. 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
1. Elevation					0.00*
Highland	150	72	78	48	a
Lowland	150	105	45	70	b
2. Village (Highland)					0.934
Serang	50	24	26	48	
Kutabawa	50	23	27	46	
Ciwarak	50	22	28	44	
3. Sex (Highland)					0.074
Cock	75	40	35	53	
Hen	75	33	42	44	
4. Village (Lowland)					0.934
Kutasari	50	33	17	66	
Kedungwuluh	50	34	16	68	
Kedungwringin	50	37	13	74	

5. **Sex (Lowland)**					0.074
Cock	75	53	22	70	
Hern	75	54	21	72	

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. trichura* 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 chicken as they are in constant contact with manure and soil (Wongrak et al., 2014) and also the eggs are resistant to the external environment (Tarbiet 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 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 prevalence between 22-84%. The prevalence level differs between locations mainly due to climatic factors, environment, and cultivation methods.

Comment [U31]: Please rephrase this sentence.

Comment [U32]: Please what does "decreased body condition" mean in this context?

Comment [U33]: Please provide some examples of such diseases with references.

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 had the ability to survive and 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. 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, 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%) (Javaredowdha 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 et al., 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).

Comment [U34]: How do these results compare with the study? Please state the relation.

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 as 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 (Yousaf et al. al., 2019). The quality of feed will greatly determine the chicken's resistance to endoparasite attacks (Subedi et al., 2020).

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, 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. trichura* species.

The results of this study recommend traditional local chicken farmers to 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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REFERENCE

Alam N , Mostofa M , Khan MHNA , Alim MA , a. K. M. A. Rahman AKMA, Trisha A. 2014. Prevalence of gastrointestinal helminth infections in indigenous chickens of selected areas of Barisal district, Bangladesh Bangl. J. Vet. Med. 12 (2): 135-139

Berhe M , Mekibib B , Bsrat A , Atsbaha G. 2019. Gastrointestinal Helminth Parasites of Chicken under Different Management System in Mekelle Town, Tigray Region, Ethiopia. Journal of Veterinary Medicine Volume 2019, Article ID 1307582, 7 pages https://doi.org/10.1155/2019/1307582

Damayanti EA, Hastutiek P, Estoepangestie ATS, Retno NR , Kusnoto, Suprihati E . 2019. The Prevalence and Infection's Degree of Gastrointestinal Worm of Local Chicken (*Gallus Domesticus*) in Kramat Village, District of Bangkalan, Madura, East Java Indonesia. Journal of Parasite Science 3 (1). https://doi.org/10.20473/jops.v3il.16436

Elele K, Amawulu E, Eniyekedidei KP. 2021. Parasites Status of Village Chickens (*Gallus gallus domesticus*) in Selected Communities in Yenagoa Local Government Area of Bayelsa State, Nigeria. Open Journal of Applied Sciences, 11, 230-238. https://doi.org/10.4236/ojapps.2021.112016

Gamraa OW, Antiab RE, Falohunc OO. 2015. Intestinal cestodes of poultry *Raillietina echinobothrida* and *Choanotaenia Infundibulum* infection in a commercial Japanese quail (*Coturnix coturnix japonica*) farm in Apomu, Osun State, Nigeria. Scientific Journal of Zoology. 4(4) 20-25 https://doi.org/10.14196/sjz.v4i4.1860

García-Cuadrado MN, F. J. Martínez-Moreno FJ, Leva RZ, García IA. 2021. Helminth communities in the alimentary tract of free raised chickens on rainfed and irrigated agrosystems from southwest Spain, Italian Journal of Animal Science, 20:1, 1689-1694. https://doi.org/10.1080/1828051X.2021.198044

Hariani N, Simanjuntak I. 2021. Prevalence and Intensity of Parasitic Worms on Free-Range Chickens and Egg-Laying Chickens in Muara Badak sub-District, Kutai Kartanegara Jurnal ILMU DASAR, 22 (1) 1-8

Idika K, Obi CF, Ezeh IO, Iheagwam CN, Njoku IN, Nwosu CO. 2016. Gastrointestinal helminth parasites of local chickens from selected communities in Nsukka region of south eastern Nigeria. J Parasit Dis. 40(4):1376–1380. https://doi.org/10.1007/s12639-015-0694-9

Imam TS , Dambo R, Sa'id MA, Suleiman K. 2017. Detection of Gastro-Intestinal Helminthes in Local Chicken (*Gallus gallus*) Sold at Sharada Market, Kano Metropolis, Nigeria . Journal Dryland Agriculture. 3(1). 19 – 27

Jaiswal K, Mishra S, Bee A. 2020. Prevalence of Gastrointestinal Helminth Parasites in *Gallus gallus domesticus* in Lucknow, U. P, India. Advances in Zoology and Botany,8(5), 422-430. https://doi.org/10.13189/azb.2020.080506

Javaregowda AK, Rani BK, Revanna SP, Udupa G .2016. Prevalence of gastro-intestinal parasites of backyard chickens *(Gallus domesticus)* in and around Shimoga. J Parasit Dis 40(3):986–990. https://doi.org/10.1007/s12639-0140620-6

Junaidu HI, Luka SA, Mijinyawa A. 2014. Prevalence of Gastrointestinal Helminth Parasites of the Domestic fowl (*Gallus-gallus domesticus*) slaughtered in Giwa Market, Giwa Local Government, Area, Kaduna State, Nigeria Journal of Natural Sciences Research. 4. (19) 120-128

Kusuma SB, Nusantoro S,Muhamad N, Awaludin A, Hasanah N, Adhytama M.2021. Identification of helminth parasite diversity on laying chicken in Jember (East Java-Indonesia). IOP.Conf.Series: Earth and Environmental Science. 672. 012045. https://doi.org/10.1088/1755-1315/i/012045

Mohammed BR, Ojo AO, Opara MN, Jegede OC, Rowland I.S. Agbede RIS. 2019. Haemo and endoparasites of indigenous chickens reared in Gwagwalada Area Council, Abuja, Nigeria. Annals of Parasitology. 65(3), 293-296 https://doi.org/10.17420/ap6503.213

Mwale M, Masika PJ. 2011. Point prevalence study of gastro-intestinal parasites in village chickens of Centane district, South Africa African Journal of Agricultural Research. 6 (9). 2033-2038, https://doi.org/10.5897/AJAR09.495

Ngongeh LA, Chiejina SN, Lawal AI. 2014. Prevalence of gastrointestinal helminth infections in slaughtered chickens reared in the Nsukka area of Enugu State, Nigeria. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS). 7, (11) 51-54

Nguyen TBV, Nguyen VC, Nguyen TP, Nguyen TH, Kiet BT, Nguyen VH, Hien VB, Thwaites G, Carrique-Mas JJ, Ribas A. 2020. Characterisation of gastrointestinal helminths and their impact in commercial small-scale chicken flocks in the Mekong Delta of Vietnam Tropical Animal Health and Production 52:53–62. https://doi.org/10.1007/s11250-019-01982-3

Ola-Fadunsin SD, Uwabujo PI, Sanda IM, Ganiyu IA, Hussain K, Rabiu M, Elelu N, Alayande MO. 2019. Gastrointestinal helminths of intensively managed poultry in Kwara Central, Kwara State, Nigeria: Its diversity, prevalence, intensity, and risk factors, Veterinary World, 12(3): 389-396.

Permin A. 2020. Impact of Helminth Infections on Production of Chickens. Biomedical. Journal of Scientific & Technical Research. 29.(3). https://doi.org/10.26717/BJSTR.2020.29.004819

Papini R, Cacciuttolo E. 2008. Observations on the occurrence of *Heterakis gallinarum* in laying hens kept on soil. Ital.J.Anim.Sci. vol. 7, 487-493.

Riwidiharso E, Darsono, Setyowati EA, Pratiknyo H, Sudiana E, Santoso S, Yani E, Widhiono I. 2020.Prevalence and diversity of ectoparasites in scavenging chickens *(Gallus domesticus)* and their association to body weight. Biodiversitas. 21(7) 3163-3169 https://doi.org/10.13057/biodiv/d210738

Rufai MA, Jato AO. 2017. Assessing The Prevalence Of Gastrointestinal Tract Parasites Of Poultry And Their Environmental Risk Factors In Poultry In Iwo, Osun State Nigeria. Ife Journal of Science. 19 (1). 2-13. https://dx.doi.org/10.4314/ijs.v19i1.2

Saraiva DJ, Campina ACC, Gonçalves FCS, Melo-Viegas D, Santos ACG, Nogueira RMS, Costa AP, Gastrointestinal Parasites in Free-Range Chicken Raised under Extensive System from the Northeast of Brazil . Brazilian Journal of Poultry Science Revista Brasileira de Ciência Avícola http://dx.doi.org/10.1590/1806-9061-2020-1337.

Sharma N, Hunt PW, Hine BC, Swick RA, Sharma NK, Ruhnke I .2017. *Ascaridia galli* challenge model in laying hens. J. Adv. Parasitol. 4(3): 41-46. DOI | http://dx.doi.org/10.17582/journal.jap/2017/4.3.41.46

Shifaw A, Feyera T, Stephen W,Brown W, Sharpe B, Elliott T, Ruhnke I .2021. Global and regional prevalence of helminth infection in chickens over time: a systematic review and meta-analysis. Poultry Science 100:101082 https://doi.org/10.1016/j.psj.2021.101082

Sherwin C M, Nasr M A, Gale E, Petek M, Stafford K, Turp M, Coles G C. 2014. Prevalence of nematode infection and faecal egg counts in free-range laying hens: relations to housing and husbandry. Br. Poult. Sci. 54(1):12-23. https://doi.org/10.1080/00071668.2012.757577

Slimane BB. 2016. Prevalence of the gastro-intestinal parasites of domestic chicken Gallus domesticus Linnaeus, 1758 in Tunisia according to the agro-ecological zones. J Parasit Dis 40(3):774–778. https://doi.org/10.1007/s12639-014-0577-5

Subedi JR, Mujahid T, Cheetri B. 2018. Prevalence of intestinal helminth parasites of chicken *(Gallus gallus domesticus* Linnaeus, 1758) in Lalitpur district, Nepal. Tribhuvan University Journal, vol. 32, no. 2,

Tanuwijaya PA, Febraldo D. Parasite Infections In Poultry Environments (Case Report On Gallus Domesticus Endoparasite) Journal of Environmental Science and Sustainable Development 4 (1). 97-136. https://doi.org/10.7454/jessd.v4i1.1083

Tarbiat B, Jansson DS, Höglund J. 2015. Environmental tolerance of free-livingstages of the poultry roundworm Ascaridia galli. Vet. Parasitol. 209: 101–107. https://doi.org/10.1016/j.vetpar.2015.01.024

Tsegaye AA, Miretie AA. 2021. Chicken Ascariasis and Heterakiasis: Prevalence and Associated Risk Factors, in Gondar City, Northwest Ethiopia. Veterinary Medicine: Research and Reports 12. 217–223. https://doi.org/10.2147/VMRR.S323284

Uhuo AC, Okafor FC, Odikamnoro OO, Onwe CS, Abarike MC, Elom JN. 2013. Common gastrointestinal parasites of local chicken (gallus domesticus) slaughtered in some selected eatery centres in Abakaliki, Ebonyi State: Implication for meat quality. International Journal of Development and Sustainability 2 (2), 1416-1422.

Vieira FEG, Yamamura MH, Freire FR, Headley SA. 2015. The effects of managerial systems on helminth infection in freerange chickens from northern Paraná, Semina: Ciências Agrárias, Londrina 36 (6) 4311-4322. http://dx.doi.org/10.5433/1679-0359.2015v36n6Supl2p4311

Win SY, Htun LL, Hmoon MM, Chel HM, Thaw YN, Soe NC, Oo HL, Bawm S. 2020. Occurrence of gastrointestinal parasites in free ranging village chickens from four townships of Myanmar. Veterinary Sciences: Research and Reviews, 6(1): 1-6. http://dx.doi.org/10.17582/journal.vsrr/2020/6.1.1.6

Wongrak K, Das G, Moors E, Sohnrey B, Gauly M. 2014. Establishment of gastro-intestinal helminth infections in free-range chickens: a longitudinal on farm study. Berlin Munich. Tierarztl. Wochenschr. 127: 314–321

Yassir Dakheel Kremsh Alasadiy , Arshad Naji Alhasnawi1 , Farhan AlaAllah Eabaid1 The Prevalence of Parasitic Infection in Domestic Chicken: A Review Medico-legal Update, January-March 2020, Vol.20, No. 1 https://doi.org/10.37506/v20/i1/2020/mlu/194434

Yousaf A, Tabasam MS, Memon A, Rajput N, Shahnawaz R, Rajpar S, Jamil T, Mushtaq M. 2019. Prevalence of *Ascaridia galli* in different broiler poultry farms of potohar region of rawalpindi pakistan. Journal of Dairy, Veterinary & Animal Research.Volume 8 Issue 1 - 2019

Yousfi F, Senouci K , Medjoual I, Djellil H, Slimane TH. 2013. Gastrointestinal helminths in the local chicken Gallus gallus domesticus (Linnaeus, 1758) in traditional breeding of North Western Algeria Biodiversity Journal. 4 (1): 229-234

Zalizar L, Winaya A, Malik A, Widodo W, Suyatno, Anggraini AD. 2021 Species identification and prevalence of gastrointestinal helminths in Indonesian native chickens, and its impact on egg production. Biodiversitas. 22(10) 4363-4369 https://doi.org/10.13057/biodiv/d221029

Title: The title of the manuscript is good and can represent the objective and findings

Generally, the study was conducted to find out the diversity and prevalence of endoparasites in domestic chickens across an elevation gradient in Central Java, Indonesia. The study is relevant as it was conducted to address a major issue facing local chicken farmers. Though this manuscript is relevant, it is currently having some major issues which need to be addressed before it can be accepted.

Major comments

1. There is no information on the methods used in the study. Methods used in the study should be included in the abstract.

2. The sentence in lines 17 and 18 "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" needs to be rephrased, as it stands now, it is grammatically incorrect and does not carry any meaning.

3. Pictures of the eggs of endoparasites found must be included in the study. Because the method of identification needs further verification.

4. The authors need to find someone who is a native English speaker to proofread the entire manuscript as it currently has many grammatical errors.

Minor Comments

1. Page 1, line 7, kindly start the sentence with "The"

2. line 8, change the word farm to farms.

3. In line 11 the word endoaparsite is wrongly spelt and should be either endoparasite or endoparasites

4. line 14, the word endoparasite should be endoparasites.

Introduction

1. Page 1, line 32, the world chicken should be changed to chickens.

Results

All issues relating to this are commented on directly in the main document

Discussion

All issues relating to this are commented on directly in the main document

Conclusion

The conclusion should have a topic on its own.