Research Article

Prevalence of Natural Gastrointestinal Helminth Infection of Thai Indigenous Chickens Aged 12–18 Weeks in Small–Scale Chicken Farms on River Plains in Central Thailand

Kunlayaphat Wuthijaree[1], Pattaraporn Tatsapong[1], Sukanya Yung-Rahang[2], Prayad Thirawong[2], Koonphol Pongmanee[2*]

[1]*Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok 65000, Thailand;* [2]*Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.*

Abstract | Helminth infection is one of the health problems in backyard chickens and is detrimental to productivity performance. This study evaluated the prevalence and average number of gastrointestinal parasites per chicken (worm burden) in Thai indigenous chickens (*Gallus gallus domesticus*). A total of, 229 chickens (113 males and 116 females) were investigated in 3 selected districts in central Thailand. Chickens were raised under extensive backyard conditions and then slaughtered at ages 12, 14, 16 and 18 weeks. Standard parasitological procedures were used to determine the worm burden in the gastrointestinal tracts. The R software application was used to compute and assess all descriptive and analytical statistics. Based on post-mortem examination of the gastrointestinal tracts, the helminths identified, three nematode species (*Ascaridia galli*, *Heterakis gallinarum*, *Capillaria* spp.), and cestodes and trematodes were found. Overall, the total prevalence of helminth infection was 79.9% (183/229), of which most were nematodes (72.1%), with a mean (± standard deviation) burden of 7.41 ± 12.81 (ranging from 0 to 78) worms per chicken. The most common helminth species identified in the examined Thai indigenous chickens were nematodes *H. gallinarum* (70.3%) and *A. galli* (14.8%). A prevalence rate of 34.5% for cestodes was observed. There was no statistically significant difference in the prevalence of helminth infections among male and female chickens. The prevalence of natural helminthic infections increases with chicken age. The results revealed that most Thai native chickens reared in backyard environments have subclinical infections with at least one helminth species.

Keywords | Helminth, Worm burden, Infection intensity, Indigenous chicken

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***Correspondence** | Koonphol Pongmanee, Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand; **Email:** koonphol.p@ku.th
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INTRODUCTION

Indigenous chickens can easily be infested with both gastrointestinal and blood parasitic pathogens (Opara *et al*., 2014; Takang *et al*., 2017). Gastrointestinal helminth parasites are a significant impediment to the productive performance of free–range and backyard chicken farming around the world (Jeni *et al*., 2021). Gastrointestinal helminths have the potential to serve as vectors for transmitting pathogens (such as *Histomonas meleagridis*) that cause avian blackhead disease (McDougald, 2005; Marchiondo *et al*., 2019). However, these helminths can serve as vectors and cause secondary infections, such as by *Escherichia coli* (McDougald, 2005; Permin *et al*., 2006) and *Salmonella* *enterica* (Shohana *et al*., 2023). Helminthiasis stands out as one of the prevalent diseases frequently encountered, affecting scavenging chickens (Soulsby, 1976). Environmental factors (for example, rainfall, humidity, and ambient temperature) can have a major impact on the occurrence of helminth infective stages (Chilinda *et al*., 2020; Van *et al*., 2020; Shifaw *et al*., 2021, Kerroucha *et al*., 2022). The parasites are ingested directly by the scavenging chickens through contaminated feed, water, or soil, or indirectly through the consumption of invertebrates (intermediate hosts) such as snails, earthworms, or others that may transport the eggs (Soulsby, 1976; Shifaw *et al*., 2021). Parasitic infections interfere with the digestive system and nutrient metabolism in chickens. The presence of worms has a negative effect on feed efficiency and overall chicken performance (Yazwinski *et al*., 2013; Van *et al*., 2020). Helminth infections can cause seriously affect chicken health and behavior (Gauly *et al*., 2007), resulting in the manifestation of both clinical and subclinical diseases, overall decreased productivity and economic loses (Gauly *et al*., 2005; Daş *et al*., 2010; Singh *et al*., 2021).

Sam Leuang, Tapaotong, and Sam Leuang × Tapaotong crossbred chickens are slow–growing indigenous breeds, commonly reared by Thai smallholder farmers using family labor under free-range, non-intensive or backyard conditions in the central Thailand. Generally, male and female indigenous chickens are collectively reared until they reach to market weight of 1.5–1.7 kg (Choprakarn and Wongpichet, 2007; Yung-rahang *et al*., 2017) at a slaughter age of 12–16 weeks (Jaturasitha *et al*., 2008; Yung-rahang *et al*., 2017). Indigenous chicken meat has a better flavor and taste than commercial broiler meat; consequently, it can fetch a higher price. Therefore, people in central Thailand continue to raise and maintain the breeding stock of indigenous chickens for local market consumption. However, the present research on gastrointestinal helminth infections in indigenous chickens in Thailand exhibits deficiencies, particularly in comprehensive data regarding prevalence and geographic distribution. Although there have been investigations into the presence of gastrointestinal helminths in indigenous chickens in the northern, northeastern, and southern of Thailand, (Kunjara Na Ayudthaya and Sangvaranond, 1993, 1997; Butboonchoo and Wongsawad, 2017), there is no published information in central Thailand. The objective of this study was to determine the infection rate of gastrointestinal helminths, identify helminth parasite species in male and female indigenous chickens of different ages, and estimate the correlation between age at slaughter and helminth infection in Thai native chickens reared in free-range backyard production systems in central Thailand. These results may provide useful epidemiological data on specific helminths in indigenous chickens in central Thailand.

MATERIALS AND METHODS

Ethical Statement

All chickens included in this study were naturally infected and all procedures performed adhered to the applicable guidance of the World Association for the Advancement of Veterinary Parasitology for Poultry. This study was carried out following the approved protocol by the Animal Care and Use Research Ethics Committee of Kasetsart University, Bangkok, Thailand (Approval No. ACKU63–AGK–002).

Study Area

The study was conducted in three selected districts in central Thailand: Kamphaeng Saen (13°59′ N; 99°59′ E) and Don Tum (13°57′ N; 100°4′E) districts, Nakhon Pathom province; and Song Phi Nong district (14°13′ N; 100°1′ E), Suphan Buri province. Both provinces are dominated by floodplains and their main agricultural products consist of rice and livestock, including chickens and pigs. The climate is tropical and characterized by hot summers (mid-February to mid-May) and cold, wet winters (mid-October to mid-February). Typically, the total annual rainfall is 127–942 mm, with the rainy season occurring take place mid-May until mid-October. The annual precipitation is 200–350 mm, reaching its highest point in August to September. The average temperature is 28.7°C, with little seasonal variation (Thai Meteorological Department, 2019). All three selected districts were in the low river plains zone at an altitude of 4.74–7.25 m above sea level. This study was conducted between June and December 2019.

Experimental Animals And Diets

In total, 229 Sam Leuang × Tapaotong one-day-old mixed-sex chicks were obtained from the Department of Animal Science, Kasetsart University, Kamphaeng Saen Campus, Thailand. All of the chick received a vaccination for Marek's disease on the first day. The chicks were randomly allocated to three groups and delivered to one small-scale chicken farm in each of the three selected study areas. At each farm, chicks were kept according to routine management practices in an optimum hygienic environment until the age of 2 weeks; subsequently, they were raised under free-range conditions. The birds were fed corn–soy-based diets providing 21% protein and 3,100 kcal/kg of metabolizable energy during the first 4 weeks and 18% protein and 2,900 kcal/kg metabolizable energy after 4 weeks. No veterinary treatments were administered during the study. The feed intake was not measured. Live body weights were recorded on the day of slaughter.

Chicken Population And Sample Size

The sample size was determined using the methodology outlined by Thrusfield (2005). Given the parasites' unknown prevalence, an approximate value of 50% was used to determine the maximum sample size. Considering a desired absolute precision of 10% with a confidence level of 95%, it was determined that a minimum sample size of 96 chickens was necessary. In total, 229 chickens were obtained after considering natural mortality and escape.

Sampling Methods And Parasitological Measurements

At 12, 14, 16, and 18 weeks of age, 15–20 birds of the same age from each farm were randomly chosen and examined for the prevalence of worms and average amount of worms per chicken (worm burden). Feed was withdrawn 10 h before slaughter but drinking water was available. On the day of slaughter, the body weight of the selected chickens was measured, the trachea and gastrointestinal tract were removed, and helminths were investigated. The gastrointestinal tract of each chicken was dissected into distinct sections, including the proventriculus, gizzard, small intestine (consisting of the duodenum, jejunum, and ileum), and cecum. Each section was longitudinally dissected and rinsed with tap water, adhering to the protocols outlined by the World Association for the Advancement of Veterinary Parasitology (Yazwinski *et al*., 2003). The intestinal mucosa and contents of the digestive tract were carefully removed to extract the adherent parasites present in the mucosa. The residue from each sample was rinsed with distilled water using a 100-μm mesh screen and then moved to a Petri dish. The remaining worms were isolated using a stereomicroscope (Leica model M26, Germany) after all visible parasites were collected. The identification of all helminth species was conducted using characteristics of morphology, as outlined by Soulsby (1976), McDougald (2008), and Yazwinski and Tucker (2008). The sex of all mature *A. galli* and *H. gallinarum* worms was identified using the approach described by Yazwinski and Tucker (2008). Additionally, the presence of helminths was examined in the proventriculus and gizzard. This study used a light microscope with magnifications ranging from 10x to 100x for cestode species identification. The identification is based on their morphological characteristics, as outlined by McDougald (2008), Yazwinski and Tucker (2008) and Soulsby (1976). The Mello-Campos approach was adapted for the cestode identification process, as described by Silva *et al*. (2016).

Statistical Analysis

The statistical analysis was performed using R software (R Core Team, 2019). The prevalence of each parasite species was determined by calculating the ratio of chickens infected with a particular parasite to the total number of chickens (Thrusfield, 2005). The prevalence of mixed helminth infections and individual helminth species was ascertained using the frequencies. Infection intensity was determined by calculating the mean number of worms harbored by a particular parasite species. To determine the sex ratio of each helminth species, the total number of female worms was divided by the number of male worms. The Kolmogorov-Smirnov test was employed to assess the normality of the distribution of quantitative variables before analysis. The correlations between different parasitological parameters within the nematode species were determined using Spearman's correlation coefficients. The data regarding the number of worms present has been analyzed using a general linear model, with the fixed effects of host sex (male versus female) and age at slaughter (12, 14, 16, and 18 weeks) and the random effects of the farm. The results are presented as mean value and standard deviation (SD). Differences in the prevalence of worm species depending on host sex and age at slaughter were analyzed using the chi-square test, and differences in worm burden were analyzed using the t-test. The confidence level was set at 95% for all analyses, and differences were considered significant at $P \leq 0.05$.

RESULTS

Body Weight, Infection Rate, And Parasite Diversity Of Indigenous Chickens

The body weights of the indigenous chickens increased with age. The mean body weight at the age of 12 weeks was 1,324 g. Body weight increased gradually at 14 weeks (1,407 g), peaked at 16 weeks (1,768 g), and remained constant until 18 weeks (1,755 g) (Figure 1). Of the 229 chickens, 183 (79.9%) were subjected to infection by at least one species of helminth. (Table 1). Nematodes were found in 72.1% of chickens, with a mean ± SD burden of 7.41 ± 12.81 (ranging from 0 to 78) worms per chicken. *H. gallinarum* emerged as the predominant nematode species, accounting for 70.3%, succeeded by *A. galli* at 14.8% and *Capillaria* spp. at 6.6%. Cestodes were detected in 34.5% of the chickens. In terms of cestodes, the prevalence of *Raillietina cesticillus*, *Raillietina echinobothrida*, *Raillietina tetragona*, *Amoebotaenia cuneata*, *Choanotaenia infundibulum*, *Hymenolepis carioca*, and *Hymenolepis cantaniana* was 29.7, 18.8, 12.2, 9.6, 9.2, 8.7, and 8.3%, respectively (Table 1). All helminths were retrieved from the gastrointestinal tract, with none identified in the trachea. Among the chickens, 45.4% exhibited infection with a single helminth species, whereas 34.5% were demonstrated infection with two or more helminth species (Table 2). The indigenous chickens exhibited no discernible clinical signs of disease throughout the study.

Prevalence Of Gastrointestinal Parasites By Sex

Table 1 displays the overall prevalence of the identified species categorized by host sex. The examination of helminth distribution within host sexes revealed that the prevalence

Table 1: Overall and host sex-dependent prevalence of helminth species (N = 229) and the odd ratios (Ψ) to show the probability of a female being infected compared with a male.

Species	Prevalence of helminth infection (%)			Gender effect (Pr > ChiSq)	Ψ
	Overall (N = 229)	Female (N = 116)	Male (N = 113)		
Nematodes	72.1	69.8	74.3	0.447	1.25
A. galli	14.8	14.7	15.0	0.934	1.03
H. gallinarum	70.3	68.1	72.6	0.460	1.24
Capillaria spp.	6.6	6.9	6.2	0.830	0.89
Cestodes	34.5	39.7	29.2	0.096	0.63
A. cuneata	9.6	10.3	8.8	0.701	0.84
H. carioca	8.7	9.5	7.0	0.684	0.83
H. cantaniana	8.3	9.5	7.1	0.510	0.73
R. cesticillus	29.7	34.5	24.8	0.108	0.63
C. infundibulum	9.2	10.3	8.0	0.533	0.75
R. echinobothrida	18.8	19.8	17.7	0.680	0.87
R. tetragona	12.2	11.2	13.3	0.633	1.21
Trematodes	4.4	5.2	3.5	0.546	0.67
Echinostoma revolutum	4.4	5.2	3.5	0.546	0.67
Total	79.9	77.6	82.3	0.373	1.34

Table 2: Prevalence of different parasites in indigenous chickens on river plains in central Thailand by genera

Parameters		Parasitic infection (%)		Nematodes	Cestodes	Trematodes
		Single infection	Multiple infection			
Age	12 weeks (N)	46.0 (23)	10.0 (5)	40.0 (20)	22.0 (11)	2.0 (1)
	14 weeks (N)	46.9 (30)	37.5 (24)	78.1 (50)	35.9 (23)	6.3 (4)
	16 weeks (N)	44.8 (26)	39.7 (23)	77.6 (45)	41.4 (24)	5.2 (3)
	18 weeks (N)	43.9 (25)	47.4 (27)	87.7 (50)	36.8 (21)	3.5 (2)
Sex	Male (N)	52.2 (59)	30.1 (34)	74.3 (84)	29.2 (33)	3.5 (4)
	Female (N)	38.8 (45)	38.8 (45)	69.8 (88)	39.7 (46)	5.2 (6)
Total (N)		45.4 (104)	34.5 (79)	72.1 (165)	34.5 (79)	4.4 (10)

Table 3: Parasitological parameters of indigenous chickens infected with gastrointestinal parasites at the age of 12, 14, 16, or 18 weeks (Mean ± SD)

		Age				*p* value
		12 weeks N=50	14 weeks N=64	16 weeks N=58	18 weeks N=57	
A. galli	Infected, % (N)	2.0[b] (1)	20.3[a] (13)	17.2[a] (10)	17.5[a] (10)	0.035
	Total worm burden	0.02±0.14	1.61±5.96	1.03±4.36	1.79±6.40	0.258
	Number of female worms	0.02±0.14	0.50±2.15	0.45±2.18	0.81±4.91	0.581
	Number of male worms	0	0.30±1.14	0.29±1.74	0.23±0.78	0.490
	Number of larvae	0	0.81±3.31	0.29±0.96	0.75±3.61	0.286

	Sex ratio (F:M)	-	0.98±1.01	0.74±0.65	6.82±14.83	0.523
H. gallinarum	Infected, % (N)	40.0^{b} (20)	73.4^{a} (47)	75.9^{a} (44)	87.7^{a} (50)	<0.001
	Total worm burden	1.30±2.25^{c}	6.86±10.58^{b}	2.81±3.15^{c}	11.95±16.54^{a}	<0.001
	Number of female worms	0.07±1.57^{c}	3.94±7.92^{b}	1.41±1.74^{c}	6.23±8.42^{a}	<0.001
	Number of male worms	0.52±1.23^{b}	2.16±3.40^{b}	0.93±1.45^{b}	4.61±7.83^{a}	<0.001
	Number of larvae	0.08±0.44^{b}	0.77±1.64^{a}	0.47±1.11^{ab}	1.11±2.54^{a}	0.011
	Sex ratio (F:M)	1.17±1.28	1.71±2.11	1.11±1.21	1.98±2.01	0.239
Capillaria spp.	Infected, % (N)	0 (0)	9.4 (6)	10.3 (6)	5.3 (3)	0.119
	worm burden	0	0.80±3.34	0.21±0.64	0.33±1.43	0.150
Nematodes	Incidence, % (N)	40.0^{b} (20)	78.1^{a} (50)	77.6^{a} (45)	87.7^{a} (50)	<0.001
	worm burden	1.32±2.27^{c}	9.27±13.53^{b}	4.05±6.08^{c}	14.07±18.07^{a}	<0.001
Tapeworms	Infected, % (N)	22.0 (11)	35.9 (23)	41.4 (24)	36.8 (21)	0.182
	worm burden	0.40±1.12	2.00±5.20	3.37±8.35	2.32±5.24	0.076
Trematodes	Infected, % (N)	2.0 (1)	6.3 (4)	5.2 (3)	3.5 (2)	0.704
	worm burden	0	0.13±0.68	2.74±18.63	0.02±0.13	0.325
total	Infected, % (N)	56.0^{b} (28)	84.4^{a} (54)	84.5^{a} (49)	91.2^{a} (52)	<0.001
	worm burden	1.77±2.55^{b}	11.19±16.08^{a}	10.05±21.86^{a}	16.40±18.82^{a}	<0.001

$^{a–c}$ means with different superscripts are significantly different (P≤0.05)

Table 4: Correlation between the different parameters in indigenous chickens infected with gastrointestinal parasites

	Age	*A. galli*	*H gallinarum*	*Capillaria* spp.	Nematodes	Cestodes	Trematodes	Worm burden
Age	-	0.12	0.36**	0.06	0.36**	0.18**	0.02	0.38**
A. galli	0.12	-	0.20**	0.52**	0.39**	0.19**	0.09	0.37**
H. gallinarum	0.36**	0.20**	-	0.19**	0.97**	0.14*	0.02	0.86**
Capillaria spp.	0.06	0.52**	0.19**	-	0.34**	0.19**	0.07	0.33**
Nematodes	0.36**	0.39**	0.97**	0.34**	-	0.19**	0.06	0.90**
Cestodes	0.18**	0.19**	0.14*	0.19**	0.19**	-	0.09	0.49**
Trematodes	0.02	0.09	0.02	0.07	0.06	0.09	-	0.18**
Worm burden	0.38**	0.37**	0.86**	0.33**	0.90**	0.49**	0.18**	-

*P≤0.05.
**P≤0.01.

of infections with diverse helminth species exhibited variations between males and females; however, there was no statistically significant distinction observed in the gender of the chickens. Of the male chickens, 52.2% had helminth infections with only one species, in contrast to 38.8% in female chickens (Table 2).

Prevalence Of Gastrointestinal Parasites By Age

The species-specific nematode prevalence was 56.0–91.2% at 12–18 weeks of age. The average worm burden was 10.2 ± 17.5. The prevalence of *H. gallinarum* was higher in chickens at 18 weeks of age than in the other age groups (P<0.001; Table 3). The same pattern was observed for nematode infections. The total nematode worm burden was higher in chickens at 18 weeks of age than in chickens of other ages (P<0.001). There was a higher prevalence of total gastrointestinal parasites in chickens at 14–18 weeks (84.0–91.2%) than in chickens at 12 weeks (56%) of age P<0.001; Table 3). Resembling trends were observed at the species level for both *A. galli* and *H. gallinarum*.

Post-mortem parasitological examinations revealed that 45.4% of chickens harboured single helminth species, while 34.5% manifested mixed infections involving at least two helminth species (Table 2). However, the prevalence of

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multiple infections at 12 weeks of age was lower than that at 14, 16, and 18 weeks of age. The prevalence of different worm species in chickens was lowest at 12, whereas it was highest at 18, weeks of age.

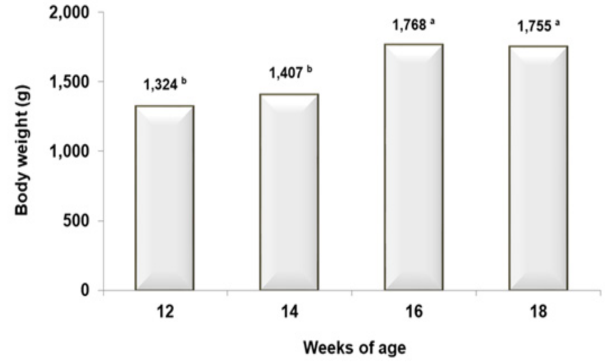

Figure 1: Average body weight of indigenous chickens at 12–18 weeks of age (different superscripts indicate significant differences at P≤0.05).

Figure 2 illustrates the incidence of multiple infections at 12, 14, 16, and 18 weeks of age. The quantity of helminth species harboured per chicken differed according to chicken age. At 12, 14, 16, and 18 weeks of age, most chickens exhibited infection with a singular helminth species. The chickens were infected with fewer helminth species at 12 and 14 weeks of age than at other ages. At 12 and 14 weeks, 25.0% and 28.2% of chickens, respectively, demonstrated mixed infections involving at least two species. Conversely, at 16 and 18 weeks, 37.9% and 40.4% of chickens, respectively, displayed mixed infections with at least two species.

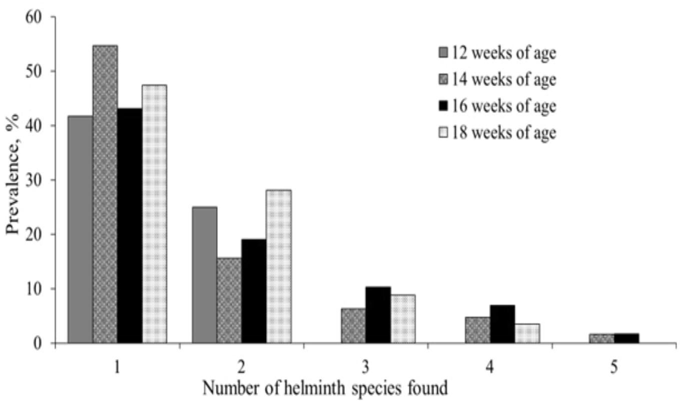

Figure 2: Distribution of the number of different helminth species found at the age of 12, 14, 16, or 18 weeks. (1=One species, 2=Two species, 3=Three species, 4=Four species, 5=Five species)

Correlations

Table 4 presents the estimated correlations among various worm species, the total worm burden, and the age of the chickens. The number of *H. gallinarum* exhibited a strong correlation (0.86) with total worm burden. The nematode count was highly correlated (0.90) with the total worm burden. Moreover, there were moderate positive correlations observed between the age of chickens and the overall worm burden. (0.38), chicken age and the number of *H. gallinarum* (0.36), and chicken age and nematode count (0.36).

DISCUSSION

There is no published information regarding the species identity, prevalence, and infection intensity of gastrointestinal helminth parasites in indigenous chickens produced on small-scale farms in the Nakhon Pathom and Suphan Buri provinces. Gastrointestinal helminths pose minimal concerns in short period of production cycle (such as broilers) reared in contemporary intensive livestock production systems; however, they continue to be a significant issue in long-cycle birds, specifically in the case of layers, particularly when reared under conditions of low biosecurity measures and inadequate sanitation (Ybañez *et al*., 2018; Ola-Fadunsin *et al*., 2019; Van *et al*., 2020). The overall prevalence of parasitic helminth infection (79.9%) reported in this study was comparatively lower than that reported in previous studies conducted in southern (83.7%; Kunjara Na Ayudthaya and Sangvaranond, 1997), northeastern (87.6%; Kunjara Na Ayudthaya and Sangvaranond, 1993), and northern (73.9%; Wuthijaree *et al*., 2019) Thailand. However, the total prevalence of helminth infection is 99.2% in the Phayao Province in northern Thailand (Butboonchoo and Wongsawad, 2017). The prevalence of natural helminth infection of the gastrointestinal tract varies regionally; it is between 97.6 and 99.2% in Germany (Kaufmann *et al*., 2011; Wongrak *et al*., 2014), 99.3% in Italy (Wuthijaree *et al*., 2017), 77.3% in Colombia (Montes-Vergara *et al*., 2021), 73.1% in Jordan (Abdelqader *et al*., 2008), 72% in Iran (Ebrahimi *et al*., 2014), and 56.43% in Indonesia (Zalizar *et al*., 2021). The probable reason for such differences in the prevalence of helminth infection might be climatic and environmental conditions, bird age, and intermediate host availability, which are the main determinants of variability in parasite prevalence. The occurrence and level of helminth infections can be impacted by temperature and humidity, affecting larvae or eggs within the environment (Ola-Fadunsin *et al*., 2019; Shifaw *et al*., 2021). In addition to environmental factors, the study season, individual host resistance, and housing management influence the prevalence rate. The indigenous chickens used in this study were housed in a free-range system. They were afforded unrestricted freedom to roam and scavenge diverse agricultural by-products, pastures, and soil. This environment exposed chickens to infective stages of parasites, including earthworms, insects, and snails, which functioned as intermediate hosts. (Yousfi *et al*., 2013).

In this study, nematodes were the most frequently reported, followed by cestodes, and trematodes. It has been suggested that the increase in the diversity and richness of helminths in indigenous chickens is linked to location, region, and climatic conditions. Temperature, humidity, and rainfall represent critical determinants impacting both the occurrence and severity of natural helminth infections (Chilinda *et al*., 2020; Van *et al*., 2020; Shifaw *et al*., 2021). Lowlands with warm temperatures, high humidity, and adequate rainfall on river plains in the Nakhon Pathom and Suphan Buri provinces may facilitate the survival of helminths in the environment, promoting the developmental success of the infective stage. Magnitude and geographical area may also affect the number of helminth species, resulting in increased helminth infections in indigenous chickens.

The most common nematode species found in the river plains was *H. gallinarum* (70.3%). According to current knowledge, no study has investigated the prevalence of natural helminth infections in indigenous Thai chickens in central Thailand. However, the prevalence of infection by *H. gallinarum* is between 70.6 (Wuthijaree *et al*., 2019) and 86.7% (Butboonchoo and Wongsawad, 2017) in northern Thailand. In contrast, the prevalence of *H. gallinarum* in southern Thailand is only 33.7% (Kunjara Na Ayudthaya and Sangvaranond, 1997). The dominance of *H. gallinarum* (60%) has been reported in the Mediterranean climates of Oran and Algeria (Kerroucha *et al*., 2022). Van *et al*. (2020) documented that the predominant helminth infection in small-scale commercial chickens in the Mekong Delta of Vietnam is *H. gallinarum*, with a prevalence of 43.3%. A study by Ybañez *et al*. (2018) of backyard chicken flocks in the Philippines revealed that the prevalence of *H. gallinarum* is 59.3%. This variation may depend on the breed and age of the chickens, housing systems, management, and environmental factors. *H. gallinarum* exhibited higher prevalence and infection intensity compared to other parasites. This could be because *H. gallinarum* is capable of infecting multiple species of galliform birds. The higher infection rates of *H. gallinarum* compared to other helminth species may be attributed to interactions with wild birds within particular environmental settings. Additionally, *H. gallinarum* exhibits both direct and indirect life cycles and can serve as a paratenic host (Soulsby, 1976; Yazwinski and Tucker, 2008), a crucial role in transmitting infective eggs in backyard farming environments. Moreover, Permin and Hansen (1998), Papini and Cacciuttolo (2008), and Yazwinski and Tucker (2008) have all noted that eggs of the cecal worm, *H. gallinarum*, are exceptionally resistant to environmental factors and can persist in the soil for years in the infectious. Although *Heterakis* spp. have a low level of pathogenicity in chickens, their significance is related to the dissemination of the protozoa *Histomonas meleagridis*, which leads to a severe re-emerging disease (histomonosis or avian blackhead disease; Daş *et al*., 2021). Previous studies by McDougald (2005) and Daş *et al*. (2021) demonstrated that the majority of *H. gallinarum* eggs obtained from hens exhibit *H. meleagridis* positivity under natural conditions.

We observed a cestode prevalence of 34.5%; the most prevalent cestode was *R. cesticillus*. In general, increased infection intensity and abundance were correlated with values of cestode prevalence. The surrounding environment is a critical determinant in natural helminth infections (Skallerup *et al*., 2005; Rufai and Jato, 2017). Poultry cestodes and trematodes have indirect life cycles. The ova typically undergo hatching in aquatic environments and undergo life cycles that involve freshwater snails or dragonflies. While the majority of species necessitate two to three intermediate hosts, a subset demands four intermediate hosts (Permin and Hansen, 1998; McDougald, 2008). The reasons for this could include geographical variations in parasite distribution or intermediate worm hosts. These results point to increased susceptibility to these parasites among the chickens kept in the wet system owing to more favorable environmental conditions, which would be associated with a greater presence of intermediate hosts. Cestodes are parasitic helminths that require intermediate hosts and optimum humidity to disseminate their susceptible components (gravid proglottids and eggs). Our findings indicate that humidity and parasite prevalence in low river plains are related, which aligns with the earlier research conducted by García Cuadrado *et al*. (2021). Their study revealed a connection between parasite prevalence and soil moisture. Moreover, the presence of swamps and, thus, a sufficient amount of intermediate hosts in the current study location might be the reason for the existence of trematodes. The findings of this study align with those of another study conducted in a location characterized by high humidity levels (Mukaratirwa *et al*., 2009).

This study did not find any correlation between parasitic infectious diseases (both the number of species and the prevalence of each species) and the sex of the host. This finding is consistent with the results of previous studies (Hussen *et al*., 2012; Ebrahimi *et al*., 2014; Butboonchoo and Wongsawad, 2017; Wuthijaree *et al*., 2019), indicating that helminth species have no natural affinity for either sex of host chicken.

The findings of this investigation demonstrated that chicken age had a variable influence on gastrointestinal parasitic infections, as chickens at 14–18 weeks of age were more likely to carry parasites than those at 12 of age. The risk of gastrointestinal helminth infections and the variety of helminth species escalated in correlation with age and access to outdoor activities. In general, the occurrence of helminth infections was significantly elevated in all husbandry systems during the period of the highest production. The increase was substantial until the time of slaughter, which aligns with the belief that the age of the host has minimal impact on resistance (Idi *et al*., 2004; Zloch *et al*., 2021). A possible reason for this could be continuous reinfection from a very contaminated surrounding environment (Höglund and Jansson, 2011; Tarbiat *et al*., 2016). The duration of host exposure to parasites influences the prevalence of natural helminth infections. As a result, backyard chickens that are raised for more extended periods and have more freedom to scavenge are likely to have a greater occurrence of gastrointestinal helminths. This is because they are more frequently exposed to these parasites' infectious stages or infected intermediate hosts (Kumar *et al*., 2015). Furthermore, more contact with contaminated environments outdoors may increase the likelihood of being reinfected. Additionally, it is crucial to consider that certain types of nematodes and cestodes rely on intermediary hosts like earthworms, houseflies, or beetles for transmission (Permin *et al*., 1999; Shifaw *et al*., 2021).

CONCLUSIONS

Gastrointestinal parasites are frequently discovered in domestic chickens raised in the backyard systems of river plains in central Thailand. Most chickens under common, extensive backyard production conditions are subclinically infected with single or multiple helminth species, the most frequent being *H. gallinarum*, Tapeworms, and *A. galli*. Increasing age correlates with a higher incidence of natural helminthic infections. The gathered gastrointestinal parasitic infection data will be useful for providing information on prevalence status and disease control and predicting future disease trends for effective farm management. Comprehending the conditions of a farm is necessary for the development of optimal practices and preventive programs, facilitating the identification of factors that impact disease incidence.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

NOVELTY STATEMENT

This paper publishes the prevalence of gastrointestinal helminth infection in free-range indigenous chickens on river plains in central Thailand for the first time. This comprehension is crucial for developing region-specific knowledge and prevention protocols regarding helminth infections in chickens with outdoor access.

AUTHORS CONTRIBUTIONS

All the authors contributed to designing research, data collection, data acquisition, data analysis and reporting, and manuscript preparation.

REFERENCES

Abdelqader A, Gauly M, Wollny CBA, Abo-Shehada MN (2008). Prevalence and burden of gastrointestinal helminthes among local chickens, in northern Jordan. Prevent. Vet. Med. 85(1-2): 17-22. https://doi.org/10.1016/j.prevetmed.2008.01.009

Butboonchoo P, Wongsawad C (2017). Occurrence and HAT-RAPD analysis of gastrointestinal helminths in domestic chickens (*Gallus gallus domesticus*) in Phayao province, northern Thailand. Saudi J. Biolog. Sci., 24(1): 30-35. https://doi.org/10.1016/j.sjbs.2015.09.002

Chilinda I, Lungu JCN, Phiri IK, Chibing OC, Simbaya J (2020). Prevalence of helminths infestation in indigenous free-ranging chickens in different ecological zones in Zambia. Livest. Res. Rural Develop., 32(9): 147.

Choprakarn K, Wongpichet K (2007). Village chicken production systems in Thailand. In: Thieme, O, Pilling, D (eds), Poultry in the 21st century: avian influenza and beyond. FAO Anim. Prod. Health Proceed.. No. 9. pp. 569-582. Rome.

Daş G, Kaufmann F, Abel H, Gauly M (2010). Effect of extra dietary lysine in *Ascaridia galli*-infected grower layers. Vet. Parasitol., 170(3-4): 238-243. https://doi.org/10.1016/j.vetpar.2010.02.026

Daş G, Wachter L, Stehr M, Bilic I, Grafl B, Wernsdorf P, Metges CC, Hess M, Liebhart D (2021). Excretion of *Histomonas meleagridis* following experimental co-infection of distinct chicken lines with *Heterakis gallinarum* and *Ascaridia galli*. Parasit. Vect., 14: 323. https://doi.org/10.1186/s13071-021-04823-1

Ebrahimi M, Asadpour M, Khodaverdi M, Borji H (2014). Prevalence and distribution of gastrointestinal helminths in free range chickens in Mashhad, northeast of Iran. Scient. Parasitolog. 15(1): 38-42.

García Cuadrado MN, Martínez-Moreno FJ, Zafra LR, Acosta GI (2021). Helminth communities in the alimentary tract of free raised chickens on rainfed and irrigated agrosystems from southwest Spain. Italian J. Anim. Sci., 20(1): 1689–1694. https://doi.org/10.1080/1828051X.2021.1980443

Gauly M, Duss C, Erhardt G (2007). Influence of *Ascaridia galli* infections and anthelmintic treatments on the behaviour and social ranks of laying hens (*Gallus gallus domesticus*). Vet. Parasitol., 146(3-4): 271-280. https://doi.org/10.1016/j.vetpar.2007.03.005

Gauly M, Homann T, Erhardt G (2005). Age-related differences of *Ascaridia galli* egg output and worm burden in chickens following a single dose infection. Vet. Parasitol., 128(1-2): 141-148. https://doi.org/10.1016/j.vetpar.2004.11.023

Höglund J, Jansson DS (2011). Infection dynamics of *Ascaridia galli* in non-caged laying hens. Vet. Parasitol., 180(3-4): 267-273. https://doi.org/10.1016/j.vetpar.2011.03.031

Hussen H, Chaka H, Deneke Y, Bitew M.(2012). Gastrointestinal helminths are highly prevalent in scavenging chickens of selected districts of eastern Shewa Zone, Ethiopia. Pakistan J. Biolog. Sci., 15(6): 284-289. https://doi.org/10.3923/pjbs.2012.284.289

Idi A, Permin A, Murrell KD (2004). Host age only partially affects resistance to primary and secondary infections with *Ascaridia galli* (Schrank, 1788) in chickens. Vet. Parasitol., 122(3): 221-231. https://doi.org/10.1016/j.vetpar.2004.04.006

Jaturasitha S, Kayan A, Wicke M (2008). Carcass and meat characteristics of male chickens between Thai indigenous compared with improved layer breeds and their crossbred. Archiv. Anim. Breed., 51(3): 283-294. https://doi.org/10.3382/ps.2006-00398

Jeni RE, Dittoe DK, Olson EG, Lourenco J, Seidel DS, Ricke SC, Callaway TR (2021). An overview of health challenges in alternative poultry production systems. Poult. Sci., 100(7): 101173. https://doi.org/10.1016/j.psj.2021.101173

Kaufmann F, Daş G, Sohnrey B, Gauly M (2011). Helminth infections in laying hens kept in organic free range systems in Germany. Livest. Sci., 141(2-3): 182-187. https://doi.org/10.1016/j.livsci.2011.05.015

Kerroucha R, Medjoual I, Bourguig L, Senouci K (2022). Identification of the main intestinal helminths of local breed chickens (*Gallus Gallus Domesticus* Linnaeus, 1758) reared in traditional mode in the Oran region. Helminthologia, 59(2): 170-178. https://doi.org/10.2478/helm-2022-0015

Kumar S, Garg R, Ram H, Maurya PS, Banerjee PS (2015). Gastrointestinal parasitic infections in chickens of upper gangetic plains of India with special reference to poultry coccidiosis. J. Parasit. Dis., 39(1): 22-26. https://doi.org/10.1007/s12639-013-0273-x

Kunjara N, Ayudthaya C, Sangvaranond A (1993). Internal parasites of alimentary tracts of adult native chickens in north-eastern part of Thailand. Agricult. Nat. Resour., 27(3): 324–329.

Kunjara N, Ayudthaya C, Sangvaranond A (1997). Internal parasites in the alimentary tracts of adult native chickens in the southern part of Thailand. Agricult. Nat. Resour., 31(4): 407-412.

Marchiondo AA, Cruthers LR, Fourie JJ (2019): Chapter 2 – Nematoda. In: Marchiondo AA, Cruthers LR, Fourie JJ (eds), Fourie Parasiticide Screening, Volume 2. Academic Press. pp. 135-335. https://doi.org/10.1016/B978-0-12-816577-5.00007-7

McDougald LR (2005). Blackhead disease (histomoniasis) in poultry: A critical review. Avian Dis. 49(4): 462–476. https://doi.org/10.1637/7420-081005R.1

McDougald LR (2008) Internal parasites. In: Saif, Y.M., Fadly, A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K., Swayne DE (eds), Diseases of poultry, 12th edn. pp. 1025-1066. Blackwell Publishing.

Montes-Vergara DE, Cardona-Alvarez J, Pérez-Cordero A (2021). Prevalence of gastrointestinal parasites in three groups of domestic poultry managed under backyard system in the Savanna subregion, Department of Sucre, Colombia. J. Adv. Vet. Anim. Res. 8(4): 606-611. http://doi.org/10.5455/javar.2021.h551

Mukaratirwa S, Hove T, Mukaratirwaa S, Hoveb T (2009). A survey of ectoparasites, cestodes and management of free-range indigenous chickens in rural Zimbabwe. J. South African Vet. Assoc., 8(3): 188-191. http://doi.org/10.4102/jsava.v80i3.200

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. Vet. World.,12(3): 389-396. http://doi.org/10.14202/vetworld.2019.389-396

Opara MN, Osowa DK, Maxwell JA (2014). Blood and gastrointestinal parasites of chickens and turkeys reared in the tropical rainforest zone of Southeastern Nigeria. Open J. Vet. Med., 4(12): 308-313.

Papini R, Cacciuttolo E (2008). Observations on the occurrence of *Heterakis gallinarum* in laying hens kept on soil. Italian J. Anim. Sci., **7(4)**: 487-493. https://doi.org/10.4081/ijas.2008.487

Permin A, Bisgaard M, Frandsen F, Pearman M, Kold J, Nansen P (1999). Prevalence of gastrointestinal helminths in different poultry production systems. Brit. Poult. Sci., 40(4): 439-443. https://doi.org/10.1080/00071669987179

Permin A, Christensen JP, Bisgaard M (2006). Consequences of concurrent *Ascaridia galli* and *Escherichia coli* infections in chickens. Acta Vet. Scand. 47(1): 43-54. http://doi.org/10.1186/1751-0147-47-43

Permin A, Hansen JW (1998). The epidemiology, diagnosis and control of poultry parasites. FAO Animal Health Manual. Food and Agriculture Organization of the United Nations. Rome.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

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 J. Sci. 19(1):7-13. http://doi.org/10.4314/ijs.v19i1.2

Shifaw A, Feyera T, Walkden-Brown SW, Sharpe B, Elliott T, Ruhnke I (2021). Global and regional prevalence of helminth infection in chickens over time: a systematic review and meta-analysis. Poult. Sci., 100(5): 101082. https://doi.org/10.1016/j.psj.2021.101082

Skallerup P, Luna LA, Johansen MV, Kyvsgaard NC (2005). The impact of natural helminth infections and supplementary protein on growth performance of free-range chickens on smallholder farms in El Sauce, Nicaragua. Prevent. Vet. Med., 69(3-4): 229-244. https://doi.org/10.1016/j.prevetmed.2005.02.003

Shohana NN, Rony SA, Ali HM, Hossain MS, Labony SS, Dey AR, Farjana T, Alam MZ, Alim MA (2023). Anisuzzaman. *Ascaridia galli* infection in chicken: Pathobiology and immunological orchestra. Immunit. Inflammat. Dis., 11(9): e1001. https://doi.org/10.1002/iid3.1001

Silva GS, Romera DM, Prado MB, Soares VE, Meireles MV (2016). Mello and Campos (1974). method adapted for the recovery of cestodes in birds (*Gallus domesticus*). Arquivos do Instituto Biológico., 83: 1-5. https://doi.org/10.1590/1808-1657000752015

Singh M, Kaur P, Singla LD, Kashyap N, Bal MS (2021). Assessment of risk factors associated with prevalence of gastrointestinal parasites in poultry of central plain zone of Punjab, India. Vet. World., 14(4): 972-977. https://doi.org/10.14202/vetworld.2021.972-977

Soulsby EJL (1976). Pathophysiology of parasitic infection. Academic Press.

Tarbiat B, Jansson DSS, Tydén E, Höglund J (2016). Comparison between anthelmintic treatment strategies against *Ascaridia galli* in commercial laying hens. Vet. Parasitol., 226: 109-115. https://doi.org/10.1016/j.vetpar.2016.07.006

Takang P, Pikulkaew S, Awaiwanont N, Numee S (2017). Prevalence and risk factors of blood parasites infection in backyard chickens in Chiang Mai. Vet. Integrat. Sci., 15(3), 157–167.

Thai Meteorological Department. The Climate of Thailand. (2019). Available at: https://www.tmd.go.th/en. (accessed 10 June 2019).

Thrusfield M (2005). Veterinary epidemiology: 3nd edn. Blackwell Science Ltd. Cambridge, Great Britain.

Van NTB, Yen NTP, Nhung NT, Cuong NV, Kiet BT, Hoang NV, Hien VB, Chansiripornchai N, Choisy M, Ribas A, Campbell J, Thwaites G, Carrique-Mas J (2020). Characterization of viral, bacterial, and parasitic causes of disease in small-scale chicken flocks in the Mekong Delta of Vietnam. Poult. Sci., 99(2): 783-790. https://doi.org/10.1016/j.psj.2019.10.033

Wongrak K, Daş G, Moors E, Sohnrey B, Gauly M (2014). Establishment of gastro-intestinal helminth infections in free-range chickens: a longitudinal on farm study. Berliner Munchener Tierarztliche Wochenschrift, 127(7-8): 314-321.

Wuthijaree K, Lambertz C, Gauly M (2017). Prevalence of gastrointestinal helminth infections in free-range laying hens under mountain farming production conditions. Brit. Poult. Sci., 58(6): 649-655. https://doi.org/10.1080/00071668.2017.1379049

Wuthijaree K, Lambertz C, Vearasilp T, Anusatsananun V, Gauly M (2019). Prevalence of gastrointestinal helminths in Thai indigenous chickens raised under backyard conditions in northern Thailand. J. Appl. Poult. Res., 28(1): 221-229. https://doi.org/10.3382/japr/pfy062

Yazwinski TA, Chapman HD, Davis RB, Letonja T, Pote L, Maes L, Vercruysse J, Jacobs DE (2003). World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the effectiveness of anthelmintics in chickens and turkeys. Vet. Parasitol., 116(2): 159-173. https://doi.org/10.1016/S0304-4017(03)00264-4

Yazwinski TA, Tucker CA (2008). Nematodes and acanthocephalans. In: Saif, Y.M. (eds), Diseases of Poultry, 12th edn. pp. 1025-1056. Blackwell Publishing, Cambridge.

Yazwinski TA, Tucker CA, Wray E, Jones L, Clark FD (2013). Observations of benzimidazole ef-ficacies against *Ascardia dissimilis*, *A. galli*, and *Heterakis gallinarum* in naturally infected poultry. J. Appl. Poult. Res., 22:75–79. http://dx.doi.org/ 10.3382/japr.2012-00606

Ybañez R.H.D., Resuelo K.J.G., Kintanar A.P.M., Ybañez A.P. (2018). Detection of gastrointestinal parasites in small-scale poultry layer farms in leyte, Philippines. Vet. World., 11(11): 1587-1591. http://doi.org/10.14202/vetworld.2018.1587-1591

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. Biodivers. J., 4(1): 229-234.

Yung-rahang S, Thirawong P, Sa-nguanpan S, Akaramathurakul P, Chaimongkol K (2017). The comparison of carcass and meat quality between the Sam Leuang, Tapaotong chickens and crossbred at 14 weeks of age. Agricult. Sci. J., 48(suppl. 2): 748-755.

Zalizar L, Winaya A, Malik A, Widodo W, 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.

Zloch A, Kuchling, S, Hess M, Hess C (2021). In addition to birds' age and outdoor access, the detection method is of high importance to determine the prevalence of gastrointestinal helminths in laying hens kept in alternative husbandry systems. Vet. Parasitol., 2021; 229: 109559. https://doi.org/10.1016/j.vetpar.2021.109559