


# Prevalence of Gastrointestinal Helminths in Thai Indigenous Chickens Raised Under Backyard Conditions in Northern Thailand

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## Primary Audience:

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### SUMMARY

This study aimed to evaluate the prevalence and worm burden of gastrointestinal parasites in Thai indigenous chickens (*Gallus gallus domesticus*) kept under extensive backyard conditions in Northern Thailand. A total of 211 male (N = 98) and female (N = 113) chickens from 11 smallholder farms were selected randomly between December 2016 and May 2017. At slaughter, fecal samples were collected to estimate fecal egg counts (presented as eggs per gram of feces) and oocyst counts (oocyst per gram of feces). The gastrointestinal tract of each animal was examined for the presence of parasites. The percentage of FEC- and FOC-positive samples was 33.7 and 55.4%, respectively. On average,  $111 \pm 328$  ascarid eggs and  $2,983 \pm 11,641$  coccidian oocysts were found. From the post mortem examination, 3 nematode species and cestodes were recovered. A total of 156 (73.9%) of the sampled chickens were infected with at least 1 helminth species. Average worm burden per chicken was 46.7 (SD = 50.9, median = 30). The most prevalent species were the nematodes *Heterakis gallinarum* (70.6%) followed by *Ascaridia galli* (60.2%) and *Capillaria* spp. (44.1%). The overall prevalence of cestodes was 27.7%. Apart from *A. galli* with higher prevalence in males than in females ( $P \leq 0.05$ ), gender did neither affect prevalence nor worm burden ( $P > 0.05$ ). Growth performance was not negatively affected by helminth infections. In conclusion, the vast majority of Thai native chickens are subclinically infected with at least 1 helminth species under the studied backyard conditions.

**Key words:** gastrointestinal parasites, prevalence, worm burden, nematodes, cestodes, Thai indigenous chickens

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## DESCRIPTION OF PROBLEM

Thai indigenous chickens (*Gallus gallus domesticus*) have ever been part of traditional live-stock farming in Thailand. These genotypes are still raised by the majority of Thai smallholder farmers under free-range or backyard conditions. Flock sizes vary largely throughout the year, mainly depending on the hatching rate, feed availability, endemic diseases, and availability of labor force of the farmers [1]. Pradu-Hangdum Chiangmai is one of the Thai indigenous genotypes and has been promoted for the purpose of conservation breeding and sustainable on-farm utilization in Northern Thailand [2]. The animals are medium sized and slow growing, and their meat is becoming more and more popular in the market, because their meat has unique taste and texture [3].

Intestinal parasites are one of the major problems in free-range and backyard chickens. Previous studies revealed that *Heterakis gallinarum*, *Ascaridia galli*, and *Capillaria* spp. are the most common species under varying production conditions [4–6]. Severe infections can cause negative effects on performance [7, 8] and nutrient utilization [9], and lead to behavioral alterations [10]. The environment (i.e., the pasture area) plays a major role for the occurrence of infective stages of helminths. The chickens pick up the parasites directly by ingesting contaminated feed, water, or litter or by eating snails, earthworms, or other insects (intermediate hosts) that can carry the eggs [11]. In Thailand, environmental conditions (e.g., rainfall, humidity, and ambient temperature) may provide favorable conditions for helminth populations. The use of indigenous genotypes, which are highly adapted to the local production environment, might be one opportunity to counteract infections.

Research on the occurrence and geographical distribution of gastrointestinal helminth infections in poultry in Thailand is, however, very limited. In typical backyard systems for meat production, male and female chickens are generally raised together until a slaughter age of 12 to 16 wk [12] when they reach a body weight of 1.0 to 1.5 kg [1]. The objective of this study was to determine the prevalence and worm burden in Thai indigenous chickens raised for meat pro-

duction under backyard conditions in Northern Thailand.

## MATERIALS AND METHODS

### *Ethical Consideration*

Animal care and handling and sampling procedures were performed by trained staff and followed animal welfare rules. All animals included in the study were naturally infected, and procedures were in line with the relevant guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) for poultry [16].

### *Study Area*

The study was conducted in 2 selected districts in Northern Thailand, namely Mae Tha and Chom Thong. Mae Tha district is located in Lamphum province (latitude: 18°27'43"N, longitude: 99°8'5"E), and Chom Thong district is located in Chiang Mai province (latitude: 18°25'2"N, longitude: 98°40'33"E). Both districts are mountainous regions with altitudes ranging between 600 and 1000 m above sea level. The climate is tropical and characterized by 3 distinct seasons: summer season from mid-February until mid-May, rainy season from mid-May to mid-October, and winter season from mid-October until mid-February. The average monthly temperature ranges from 17.5°C in December to 44.5°C in April. The mean annual relative humidity is 71%, and the mean annual rainfall is 1,067 mm [13].

### *Study Population and Management*

The study was conducted from December 2016 to May 2017. A total of 211 male (N = 98) and female (N = 113) Thai indigenous chickens (*Gallus gallus domesticus*) of the specific genotype named Pradu-Hangdum Chiangmai were randomly collected from 11 smallholder farms at the end of the fattening period, which varied from 12 to 15 wk of age. A sample of birds of each farm was recorded at slaughter. The animals originated from eggs that were collected from 2 flocks and hatched by a hatchery at the Lamphun

College of Agriculture and Technology, Thailand. These flocks of 15 hens each (5 per sire) were scavenging under free-range conditions. They were representing one breeding population and without any incidence of crossbreeding with exotic breeds. Starting from the first day of life, chicks were kept under the routine management practice and optimum hygienic environment until 2 wk of age before they were brought to the 11 smallholder farms. At the farms, animals of different age groups were raised together under backyard conditions. During the daytime, the animals were allowed to scavenge for edible insects, seeds, and pasture. During the night, they were kept in small shelters made from bamboo. The chicken received commercial broiler diets (175 g crude protein/kg and 12.13 MJ metabolizable energy/kg) mixed with broken rice once or twice a day. Feed intake was not recorded. No vaccinations and other anthelmintic or anticoccidial treatments were applied. The average number of chickens per household varied between 50 and 100 birds.

Sample size was calculated according to Thrusfield [14]. As the prevalence of parasites was unknown, each was assumed to be 50% where the maximum sample size is calculated. With a desired absolute precision of 10 and 95% level of confidence, a sample size of at least 96 birds was required.

### *Parasitological Examination*

**Fecal Egg Counts** Individual fecal samples were collected during the slaughter process either as freshly dropped feces or from the colon. The individual fecal samples were analyzed to estimate the number of eggs per gram of feces (EPG) using a modified McMaster counting technique with a sensitivity of 50 EPG [15]. As the eggs of *A. galli* and *Heterakis* spp. are similar in morphology to be clearly differentiated, they were counted together and are named as ascarid eggs in the following.

**Worm Burdens** Immediately after slaughter, the gastrointestinal tract was removed and separated into esophagus, crop, proventriculus, gizzard, small intestine, and ceca. Each part was opened longitudinally and its contents were flushed with tap water through a 100- $\mu$ m mesh sieve and then transferred to Petri dishes. Vis-

ible helminths were collected, and the contents of the gastrointestinal tract and the scraped mucosa were inspected under a stereomicroscope. The mucosa of the intestine was scraped, and the keratinized layer of the gizzard was peeled off to collect the helminths embedded in the mucosal layer. The procedures followed the recommendations of the WAAVP guidelines for evaluating the effectiveness of anthelmintics in chickens and turkeys [16]. The helminth species were identified according to the description given by Soulsby [11]. Scolices of cestodes were not collected, and species were not further differentiated. After identification, the numbers of adult worms and larvae were counted and sexed. Up to 10 female and 10 male worms per bird were randomly selected and measured for length under a stereomicroscope.

### *Statistical Analysis*

All descriptive and analytical statistics were conducted using SPSS (version 23). The Kolmogorov–Smirnov test was applied to test for normal distribution. Variables such as number of male and female worms, total worm count, and egg excretion rate, which were not normally distributed, were analyzed with non-parametric tests. Prevalence for each worm species was calculated as the percentage of chickens positive for this specific worm species. Infection intensity was calculated as the average number of worms per infected host. For each nematode species, sex ratio was calculated by dividing the number of female by that of male worms. Differences for the prevalence of each worm species between the host sexes were analyzed with the chi-square test. General linear models (GLM) were used to determine the effect of host sex on parasitological parameters (worm counts of each species and total worm burden) with farm as random factor including age and body weight at slaughter as covariables. Correlations between mean worm burdens, body weights, and age of birds at sampling were analyzed by Spearman's rank correlation coefficients.

To test for differences of parasite-specific prevalence between sexes, the chi-square test was used. The effect of infection on growth performance was estimated by calculating the average daily weight gain (ADG) during the last

2 wk preceding slaughter. Infection status was coded as infected, if at least 1 worm was identified per animal, or as uninfected. This was done separately for *A. galli*, *H. gallinarum*, *Capillaria* spp., overall helminths, and coccidia per examined host. The effect of the infection status on ADG was then analyzed with the GLM procedure including infection status, host sex, and its interaction as fixed effects and farm as random effect.

## RESULTS

### Body Weights

Age and body weight at slaughter of local chicken in each farm are shown in Table 1. Slaughter ages ranged from 80 to 101 d of age and body weights, expressed as average per farm, from 748 to 1,172 g.

### Fecal Egg and Oocyst Counts

Overall, FEC ranged from 0 to 1,641, whereas 33.7% of the feces samples were FEC positive ( $\geq 50$  EPG; Table 2). The mean value for ascarid eggs was  $110.8 \pm 327.9$  EPG, while only

3 samples exceeded the threshold of 1,000 eggs. *Capillaria* eggs were found in 13.5% of the samples. In 55.4% of the samples, coccidian oocysts with a mean value of  $2,983 \pm 11,641$  oocyst per gram of feces (OPG) were found (Table 2).

### Postmortem Analysis

A total of 156 (73.9%) of the sampled chickens were infected with at least 1 helminth species. Out of the infected animals, 72.5% were infected with nematodes, while cestodes were found in 27.7% (Table 3). No parasites were recovered from esophagus, crop, proventriculus, and gizzard. Of the infected chickens, 7.6% were infected with only 1 helminth species, while 19.0% were infected with 2 and 32.7% with 3 species. Almost 15% of the birds were infected with 4 species.

The chickens harbored an average of  $46.7 (\pm 4.1)$  worms per bird with the highest number for *H. gallinarum* ( $35.2 \pm 3.0$  worms), followed by *Capillaria* spp. ( $18.7 \pm 2.9$  worms) and *A. galli* ( $3.1 \pm 0.3$  worms). The sex ratio of *A. galli* was 0.67 and 1.15 in *H. gallinarum*. The average worm length for female and male *A. galli* worms

**Table 1.** Number and Age of Chickens, Average Body Weight at Slaughter and Worm Burdens of Thai Native Chickens of 11 Smallholder Farms in Northern Thailand.

Farm	N	Age at slaughter (days)	Average body weight (g) ( $\pm$ SD)	Mean worm burden ( $\pm$ SD)
1	20	80	1,062 $\pm$ 97.3	117.1 $\pm$ 74.4
2	20	92	1,172 $\pm$ 162.7	8.3 $\pm$ 5.5
3	22	101	1,159 $\pm$ 208.4	32.6 $\pm$ 35.3
4	19	93	1,070 $\pm$ 188.1	0
5	18	85	874 $\pm$ 132.7	60.2 $\pm$ 32.6
6	19	93	1,132 $\pm$ 204.9	8.9 $\pm$ 6.5
7	20	85	858 $\pm$ 110.2	0
8	27	86	934 $\pm$ 165.8	52.9 $\pm$ 37.8
9	20	92	967 $\pm$ 143.9	59.9 $\pm$ 34.5
10	20	92	825 $\pm$ 101.1	1.0 $\pm$ 0
11	6	92	748 $\pm$ 139.3	4.2 $\pm$ 2.6
Total/average	211	90	998 $\pm$ 199.8	46.7 $\pm$ 50.9

**Table 2.** Descriptive Statistics (Number and Percentage of Positive Samples and Mean, SD, Min, and Max) of Fecal Egg/Oocyte Counts in Thai Native Chickens of 11 Smallholder Farms in Northern Thailand (N = 74).

Species	No. positive	%	Mean	SD	Min	Max
Ascarid eggs	20	27.0	110.8	327.9	0	1,641
<i>Capillaria</i> spp. eggs	10	13.5	37.2	128.3	0	741
Coccidian oocysts	41	55.4	2,983.2	11,641.1	0	92,267

**Table 3.** Prevalence of Helminth Infections (Percentage of Infected Birds) in Male and Female Thai Native Chickens of 11 Farms in Northern Thailand.

Helminth species	Total (N = 211)	Sex		Effect of gender ( <i>P</i> value)
		Male (N = 98)	Female (N = 113)	
<i>Ascaridia galli</i> (adult worms)	37.0	51.0	24.8	0.024
<i>Ascaridia galli</i> (only larvae)	23.7	18.4	28.3	0.090
<i>Heterakis gallinarum</i> (adult worms)	69.2	72.4	66.4	0.397
<i>Heterakis gallinarum</i> (only larvae)	1.4	1	1.8	0.646
<i>Capillaria</i> spp.	44.1	46.9	41.6	0.435
Total nematodes	72.5	77.6	68.1	0.129
Tapeworms	27.7	26.5	28.3	0.770

**Table 4.** Average Number of Worms per Hen (Mean  $\pm$  SE), Sex Ratio and Average Length of *A. galli* and *H. gallinarum* in Male and Female Thai Native Chicken of 11 Farms in Northern Thailand.

	Male (N = 76)	Female (N = 76)	Total (N = 152)	Effect of gender ( <i>P</i> value)
<i>Ascaridia galli</i> , total, <i>n</i>	3.4 $\pm$ 3.0	2.3 $\pm$ 2.5	3.1 $\pm$ 0.3	0.003
Male, <i>n</i>	1.4 $\pm$ 1.4	1.0 $\pm$ 1.2	1.3 $\pm$ 0.2	0.192
Female, <i>n</i>	2.0 $\pm$ 2.2	1.3 $\pm$ 2.0	1.7 $\pm$ 0.2	0.007
Larvae, <i>n</i>	10.6 $\pm$ 19.9	7.2 $\pm$ 9.0	8.9 $\pm$ 1.3	0.290
Sex ratio, (female: male worms)	0.84 $\pm$ 0.27	0.40 $\pm$ 0.61	0.67 $\pm$ 0.1	0.064
Length (mm), M	40.7 $\pm$ 20.4	27.0 $\pm$ 15.8	37.0 $\pm$ 21.4	
Length (mm), F	53.8 $\pm$ 29.5	42.4 $\pm$ 24.0	42.8 $\pm$ 27.5	
<i>Heterakis gallinarum</i> , total, <i>n</i>	41.0 $\pm$ 40.2	30.2 $\pm$ 32.4	35.2 $\pm$ 3.0	0.185
Male, <i>n</i>	20.2 $\pm$ 20.0	14.8 $\pm$ 15.8	17.4 $\pm$ 1.5	0.270
Female, <i>n</i>	20.8 $\pm$ 20.9	15.4 $\pm$ 16.9	18.0 $\pm$ 1.6	0.209
Larvae, <i>n</i>	11.7 $\pm$ 18.7	8.9 $\pm$ 11.3	10.1 $\pm$ 1.2	0.415
Sex ratio, (female: male worms)	1.16 $\pm$ 0.19	1.15 $\pm$ 0.85	1.15 $\pm$ 0.1	0.460
Length (mm), M	8.2 $\pm$ 1.6	9.3 $\pm$ 1.7	8.2 $\pm$ 1.8	
Length (mm), F	9.7 $\pm$ 1.9	10.5 $\pm$ 3.0	9.7 $\pm$ 2.4	
<i>Capillaria</i> spp.	18.4 $\pm$ 23.5	18.9 $\pm$ 27.2	18.7 $\pm$ 2.9	0.941
Overall nematodes	62.2 $\pm$ 56.5	50.3 $\pm$ 44.9	46.7 $\pm$ 4.1	0.321

was 42.8 and 37.0 mm and 9.7 and 8.2 mm for *H. gallinarum*, respectively (Table 4).

### Correlations

The estimated correlations between different worm species and total worm burden of chickens are given in Table 5. The number of *H. gallinarum* was highly correlated ( $r = 0.96$ ) with total worm burden. Besides, the number of all other species was positively correlated.

### Effect of Host's Sex on Prevalence and Worm Burdens

The total prevalence did not vary between sexes ( $P > 0.05$ ), while males showed a higher prevalence of *A. galli* compared to the females ( $P \leq 0.05$ ) (Table 3). The number of differ-

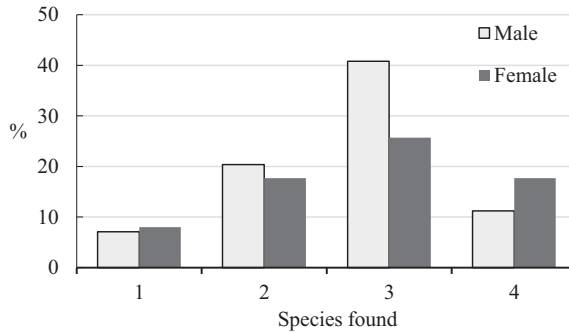
ent helminth species per chicken separated by sex is shown in Figure 1. The number of harbored helminth species per hen differed between sexes. In males, 72.4% ( $N = 71$ ) had a mixed infection with at least 2 species, while it was 61.1% ( $N = 69$ ) in females. Males (40.8%) harbored 3 species more often than females (25.7%) (Figure 1).

### Effect of Infection Status on Body Weight

The effects of parasitic infection on the final body weights at slaughter are presented in Table 6. The infection status in terms of *A. galli*, *H. gallinarum*, and overall nematodes and coccidia, which was coded as infected if at least one parasite of the different species was found per examined host, did not affect the final body

**Table 5.** Correlations Between Different Worm Species and Total Worm Burden of Thai Native Chicken of 11 Smallholder Farms in Northern Thailand (N = 152).

	<i>A. galli</i>	<i>H. gallinarum</i>	<i>Capillaria</i> spp.
<i>Ascaridia galli</i>	-	0.48**	0.33**
<i>Heterakis gallinarum</i>	0.48**	-	0.60**
<i>Capillaria</i> spp.	0.33**	0.60**	-
Total worm burden	0.52**	0.96**	0.74**

\*  $P \leq 0.05$ .\*\*  $P \leq 0.01$ .**Figure 1.** Distribution of the number of different helminth species found in male and female Thai native chicken of 11 smallholder farms in Northern Thailand.**Table 6.** Effects of Parasitic Infection (Infected vs. Uninfected) and Host Sex and Its Interaction on the Average Final Body Weight (g) (Mean  $\pm$  SD) at Slaughter in Thai Native Chicken of 11 Farms in Northern Thailand.

Species	Male (N = 98)		Female (N = 113)		<i>P</i> -value		
	Infected (n = 76)	Uninfected (n = 22)	Infected (n = 76)	Uninfected (n = 37)	Infected	Sex	Interaction
<i>Ascaridia galli</i>	1,129 $\pm$ 184.2	1,049 $\pm$ 229.4	932 $\pm$ 175.5	913 $\pm$ 142.1	0.766	0.001	0.585
<i>Heterakis gallinarum</i>	1,110 $\pm$ 194.0	1,038 $\pm$ 244.8	934 $\pm$ 147.9	886 $\pm$ 152.5	0.545	0.001	0.822
<i>Capillaria</i> spp.	1,055 $\pm$ 176.6	1,121 $\pm$ 233.7	900 $\pm$ 151.1	930 $\pm$ 149.9	0.573	0.001	0.432
Overall nematodes	1,118 $\pm$ 203.2	993 $\pm$ 210.6	931 $\pm$ 148.9	890 $\pm$ 152.1	0.557	0.005	0.911
Coccidia	1,151 $\pm$ 232.1	999 $\pm$ 235.0	871 $\pm$ 137.4	978 $\pm$ 153.8	0.157	0.001	0.330

weights, which was about 150 g higher in males than in females ( $P \leq 0.001$ ).

## DISCUSSION

The results from this study showed that gastrointestinal nematodes were prevalent in the monitored Thai indigenous chickens kept under smallholder conditions in Northern Thailand. When evaluating results of the present studies, it first has to be considered that body weights at slaughter were low. In 6 out of 11 farms, the final weight was lower than usual, because birds probably suffered from heat stress and due to parasite infection, which in turn resulted in reduced feed intake and an altered performance [17]. In com-

parison to previous studies from Thailand, the prevalence of helminth infections observed in the present study was lower. For example, there are reports of 87.6% infected chickens at 1 to 3 yr of age in North-eastern [18], 83.7% infected chickens at ages of 3 to 8 mo in Southern [19], and 99.2% in adult chickens in Northern Thailand [20]. Previous studies from other regions in laying hens, which were examined at the end of laying period and thus at a much older age compared to the present study, reported varying prevalence rates. Rates varied between 97.6 and 99.2% in Germany [5, 21], 99.3% in Italy [6], 72% in Iran [22], and 73.1% in Jordan [4]. Thus, the prevalence of helminth infections varied between regions and countries and can be mainly



related to management- and environment-related factors. Possible explanations for varying prevalence rates may be that the cited studies were conducted at different seasons, at different ages of the animals, in environments with varying availability of intermediate hosts, with animals of different individual host resistance, and under varying environmental conditions [23]. In addition, under natural helminth infection the exposure duration of hosts to parasites affects prevalence. Consequently, higher prevalence of gastrointestinal helminths in free-range or backyard chickens is expected because fattening periods are generally longer and are allowed for scavenging during most of the time, and thus are more frequently exposed to infective stages/infected intermediate hosts of the helminths compared with floor husbandry systems [24]. Although gastrointestinal helminths of free-range chickens are widely distributed throughout the world, the rate of infection is particularly common in tropical and subtropical regions [25] and in regions with high rainfall [26].

The fact that *H. gallinarum* and *A. galli* were the most frequent species of helminths widely agrees with previous reports from different regions of Thailand [20] and also from other regions of the world [5, 21]. This can be explained by the fact that both nematodes can complete their life cycle without intermediate hosts [11]. Furthermore, the number of animals being infected with multiple helminth species in the current study was lower compared to the above-mentioned reports. This difference might be associated with variations in the environmental conditions and the geographical distribution of helminth parasites and their intermediate hosts [27]. In general, the development of the different stages of helminths in the environment depends on ambient temperature and adequate moisture, because they are transmitted by invertebrate intermediate hosts that are abundant in the tropics and whose control is very limited [26]. Additionally, increasing temperature due to changing climatic conditions may have affected the occurrence of helminth infections [20]. Besides, the fact that the average worm burdens of the different species in this study were low may be due to hot and dry weather during the sampling period. Consequently, parasite abundance might be higher from midsummer onward when rainfall

increases. However, our findings clearly demonstrate that infective parasite stages remain on pasture areas throughout the hot and dry weather conditions in summer. The ability of the free-living stages to persist in the environment during summer conditions is an adaptation that has evolved in this parasite in order to survive in the environment in which its host resides [28]. Given that exposure to infective parasite stages can therefore be expected to be higher during the rainy season, strategies to combat infections are needed year round under tropical conditions.

In accordance with the observations of previous studies [20, 22, 29], the prevalence of helminth infections did not differ between male and female chicken, except for *A. galli*. Other studies reported that male birds are more susceptible to natural or experimental nematode infections than female birds [4, 30]. Gauly et al. [30] suggested that males generally exhibit reduced immune responses as well as an increased intensity and prevalence of infections compared to females. In addition, differences between females and males in susceptibility to parasite infections are probably caused by a difference in behavior, morphology, or physiological status [31].

With regard to the effects of the infection on chicken growth performance, decreased weight gains during the last 2 wk preceding slaughter were not found. This implies that infection intensity of either helminths or coccidia was low and did not result in clinical signs. It also has to be considered that the infection status was only recorded at slaughter without any knowledge at which stage of the fattening period infections occurred. Clearly, further studies are warranted to validate these findings and estimate thresholds for negative effects of parasite infections on growth performance, which may differ in Thai native or other autochthonous chicken breeds in comparison to other high-performing genotypes. Only severe infections with coccidia can produce a retardation of growth and stunting in young animals [32], and according to McDonald [33], this is achieved with extremely large infection doses ( $10^4$ – $10^6$  oocysts per bird). Therefore, it can be assumed that infections of up to 3,000 OPG as found in the present study do not affect growth performance of the host animal. Infections with multiple helminth species was a very common phenomenon. This is well in agreement

with the mixed infections in free-ranging chickens in several studies [5, 34]. On the one hand, the environmental conditions and free-range management systems are favorable for the simultaneous development of various helminth species [35]. On the other hand, backyard or free-range chickens scavenge for food in environments where various insects that may act as intermediate hosts/vectors are abundant.

## CONCLUSIONS AND APPLICATIONS

1. The vast majority of Thai indigenous chicken under the common extensive backyard production conditions were subclinical infected with multiple helminth species even when slaughtered for meat production at young ages.
2. Parasite infections, however, did not affect chicken growth performance.

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