

Original research article

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PREVALENCE OF GASTROINTESTINAL HELMINTHS IN FREE-RANGE POULTRY (*GALLUS GALLUS DOMESTICUS*) IN THE NORTHEAST OF TUNISIA

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Abstract

This study was carried out to identify and estimate the prevalence of intestinal helminths in free-range poultry (*Gallus gallus domesticus*), from September 2016 to December 2022. A total of 494 birds of both sexes, aged from 6 weeks to 36 weeks, were examined and necropsied. Mucosa and contents of different gastrointestinal tract segments were examined for the helminth parasites. Results showed that 38.86% (192 birds), as overall prevalence, were infected by at least one parasite species. The prevalence of gastrointestinal helminth infections was higher in young animals (under 18 weeks) (32.79%) than adults (6.07%) ($p < 0.05$) and during rainy and cold seasons (autumn: 59.18%; spring: 41.72%; summer: 30.7%; winter: 28.84%) ($p < 0.05$). Nematodes were more prevalent (30.97%) than cestodes (21.66%). The prevalence was significantly higher in young chickens compared to adult animals ($p < 0.05$). Among the helminths species, *Raillietina* genus was the most observed (22.67%) (*Raillietina echinobothrida*: 21.05%; *Raillietina* spp: 1.62%) followed by *Ascaridia galli* (21.25%), *Heterakis gallinarum* (12.35%) and *Capillaria* spp. (4.86%). To our knowledge, this is the first report of gastrointestinal helminths prevalence in free-range chickens

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in the Northeast of Tunisia. Further additional studies are needed to develop better preventive measures in free-range poultry flocks.

Key words: backyard poultry, nematode, cestode, prevalence, Tunisia

PREVALENCA GASTROINTESTINALNIH HELMINTA KOD SLOBODNO DRŽANE ŽIVINE (*GALLUS GALLUS DOMESTICUS*) U SEVEROISTOČNOM TUNISU

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Kratak sadržaj

Istraživanje je sprovedeno u cilju identifikovanja i procene prevalencije intestinalnih helminta kod slobodno držane živine (*Gallus gallus domesticus*) u periodu od septembra 2016. do decembra 2022. Obdukovano je i ispitano ukupno 494 uginulih jedinki oba pola, starosti od 6 do 36 nedelja. Sluzokoža i sadržaj iz različitih gastrointestinalnih segmenata su ispitani na prisustvo helmintskih parazita. Rezultati su pokazali da je 38.86% (192 jedinki), u smislu ukupne prevalencije, bilo inficirano bar jednom vrstom parazita. Prevalencija gastrointestinalnih helmintskih infekcija bila je veća kod mladih jedinki (starosti ispod 18 nedelja) (32.79%) nego kod starijih (6.07%) ($p < 0.05$) kao i tokom kišnih i hladnih perioda (jesen: 59.18%; proleće: 41.72%; leto: 30.7%; zima: 28.84%) ($p < 0.05$). Viši stepen prevalencije utvrđen je za nematode (30.97%) u poređenju sa cestodama (21.66%). Od svih vrsta helminta, najčešće su uočeni oni iz roda *Raillietina* (22.67%) (*Raillietina echinobothrida*: 21.05%; *Raillietina* spp: 1.62%), a zatim *Ascaridia galli* (21.25%), *Heterakis gallinarum* (12.35%) i *Capillaria* spp (4.86%). Prema informacijama koje posedujemo, ovo je prvo istraživanje prevalencije gastrointestinalnih helminta kod slobodno držane živine u severoistočnom

Tunisu. Neophodne su dalje studije u cilju razvoja uspješnijih preventivnih mera u jatima u slobodnom uzgoju.

Ključne reči: slobodno držana živina, nematode, cestode, prevalencija, Tunis

INTRODUCTION

The traditional poultry sector holds significant socio-economic importance in rural areas of Tunisia. While it is a major source of income for many families or even villages, free-range poultry only contributed an average of 7% to the national production economy between 2006 and 2010. About 2.773 million layer hens and 1.349 million meat chickens constitute the backyard poultry flocks, according to the last official national estimates in 2010 (GIPAC, 2010). However, the free-range poultry sector contributes to the consumption and supplies local markets for eggs and meat. In this way, free-range chickens participate at least 12% and 9.66% in the national production of poultry meat and eggs, respectively.

However, in many cases, the birds' productivity can be improved to use the bird's full genetic potential. The traditionally reared poultry farming system exposes chickens to serious health problems because of malnutrition, poor management, lack of biosecurity conditions, failure of vaccination programs, poor housing, and lack of specific veterinary care services. Furthermore, poor genetic potential due to lack of selection and predators pose potential threats to productivity. The backyard chicken feed on a wide range of food substances, varying from grains, fruits, and insects, which may harbor infective stages of parasites, thereby predisposing them to parasitic infection. In this context, parasitic infection is an important health problem in free-range poultry, which handicaps production performances (Mungube et al., 2008).

A central problem of the backyard system is the fact that birds are exposed to a constant risk of infection, mainly cestodes and nematodes, due to direct contact with the feces, the food, the water, and paratenic and intermediate hosts (Permin et al., 2002; Nyoni and Masika, 2012). In addition, a number of epidemiological factors, including the host, sex, age, breed, and environment, may influence the occurrence and the intensity of parasitic infestations (Nadeem et al., 2007).

Helminth infections are usually associated with diarrhea, nutriment spoilage, reduction in growth rate, emaciation, and decreased egg production.

An increased worm burden can even cause the death of animals. In addition, parasites are biological vectors transmitting a number of pathogens. In this way, *Heterakis gallinarum* is considered a potential vector of *Histomonas meleagridis*, which causes blackhead disease in many galliform birds (Soulsby, 1982; Ruff, 1999; Salam, 2015).

Investigating gastrointestinal helminth infections in free-range chickens is crucial to understand the epidemiological status in backyard flocks and define control measures. However, very few scientific reports on the prevalence of helminth infections in backyard chickens in Tunisia have been published (Ben Slimane, 2016). Therefore, this study aimed to investigate the occurrence of gastrointestinal helminths in free-range chickens raised under the traditional system and to identify the most common parasite species.

MATERIAL AND METHODS

Study area and sampling

This study was carried out on 494 dead backyard chickens from September 2012 to June 2018. Animals originated from farms reared under traditional conditions and located in regions around the town of Sidi Thabet in the Ariana Governorate, Tunisia. Sidi Thabet is located northeast of Tunis city center, around 36°51'45"N 10°11'44"E with a hot-summer Mediterranean climate, where winters are mild with moderate rainfall, and summers are hot and dry. Temperatures in July and August can exceed 40 °C (104 °F). Winters are mild, with temperatures rarely exceeding 20 °C (68 °F).

Sample examination

All dead backyard chickens (free-range poultry) were from farms reared under traditional conditions, were of both sexes and aged 6 to 36 weeks. They were classified into young (≤ 18 weeks) and adult animals (> 18 weeks). All the birds were necropsied in the Avian Clinic of the National Veterinary Medicine School of Tunisia and a postmortem examination was performed. After postmortem examination, the gastrointestinal tract was removed, and different portions were opened and rinsed with a physiological saline solution. Mucosa and contents of all segments were placed in Petri dishes containing water in order to be examined under a binocular magnifying glass with a black background surface (Stemi 305-Zeiss) and optic microscope for the research of adult helminths and their eggs. Parasites were identified according to their morphological characteristics using the key criteria mentioned by Soulsby (1982).

Statistical analysis

Comparative analysis of prevalence in chickens was performed using the Chi-deux test (Word Excel, Microsoft office, version 2016). A p -value < 0.05 was considered statistically significant.

RESULTS

All helminth parasites were collected from the intestines and caeca. However, no helminths were observed in the esophagus, crops, proventriculus, and gizzard. The overall prevalence of helminth infections was 38.86% of the chickens examined (494 birds).

Helminth parasites were identified in broilers, pullets, layers, and roosters (Table 1). However, pullets (50.63%) and broilers (31.16%) were most frequently affected, followed by layers (25.71%) and roosters (14.28%) ($\chi^2 = 28.46$; $p < 0.05$).

Table 1. Prevalence of gastro-intestinal helminths in free-range poultry (n = 494) according to the season and the type of production

		No. examined	No. positive	Prevalence (%)	χ^2 (p -value)	Overall prevalence
Season	Autumn	98	58	59.18%	26.94 ($p < 0.001$)	38.86%
	Winter	156	45	28.84%		
	Spring	139	58	41.72%		
	Summer	101	31	30.7%		
Type of production	Broilers	138	43	31.16%	28.46 ($p < 0.001$)	
	Pullets	237	120	50.63%		
	Layers	105	27	25.71%		
	Roosters	14	2	14.28%		

The study, according to the season, showed that helminth infections were diagnosed throughout the year, with highest prevalence noted during autumn (September, October, November) (59.18%), spring (march, April, May) (41.72%), and secondly during summer (June, July, August) (30.7%) and winter (December, January, February) (28.84%) (Table 1) ($\chi^2 = 26.94$; $p < 0.05$).

The prevalence related to the sex of birds showed helminth infections in 25.50% of females and 13.36% of males. The sex had no significant influence on the distribution of infected birds ($\chi^2 = 2.389$; $p > 0.05$) (Table 2). Accord-

ingly, a higher prevalence of helminth infections was observed in young birds (under 18 weeks) (32.80%) than in adults (> 18 weeks) (6.07%). The influence of age was statistically significant ($\chi^2 = 12.304$; $p < 0.05$).

Table 2. Prevalence of gastro-intestinal helminths in free-range poultry (n = 494)

		No. ex- amined	Nematodes		χ^2 (<i>p</i> -value)	Cestodes		χ^2 (<i>p</i> -value)
			No. posi- tive	Prevalence (%)		No. posi- tive	Preva- lence (%)	
Age	Young	375	133	35.46%	14.712	92	24.54%	7.575
	Adults	119	20	16.80%	(<i>p</i> =0.00013)*	15	12.60%	(<i>p</i> =0.006)*
Sex	Male	150	52	34.67%	1.376	31	20.67%	0.125
	Female	344	101	29.36%	(<i>p</i> =0.240)	76	22.09%	(<i>p</i> =0.723)

* significant difference

Among the total of animals necropsied, 41.67% (80 birds) and 20.33% (40 birds) had nematode and cestode infections, respectively. A significant finding was that 37.5% (72 birds) had mixed infections of nematodes and cestodes, with young animals and females being the most infected in each category with significant prevalence ($p < 0.05$).

The prevalence of nematode infections was 30.97% (153 animals). The infection was higher in young (35.46%) and in male birds (34.67%) than in adults (16.80%) and females (29.36%) ($p < 0.05$) (Table 2). Findings of cestode infections indicate an overall prevalence of 21.66% (107 animals). A significant prevalence was observed in young birds (24.54%) than in adults (12.60%) ($p < 0.05$). However, a higher prevalence of cestode infections was noted in females (22.09%) than in males (20.67%).

Different species of intestinal helminths were identified in this study (Table 3). Parasitological investigations showed that *Ascarida galli* (105 birds) (21.25%) (Figure 1A and Figure 2) was the most prevalent parasite followed by *Raillietina echinobothrida* (104 birds) (21.05%) (Figure 1B), *Heterakis gallinarum* (61 birds) (12.35%) (Figure 3 and Figure 4), and *Capillaria* spp (24 birds) (4.86%) (Figure 5).

Table 3. Prevalence of individual parasites in female, male, young (≤ 18 weeks) and adult (> 18 weeks) free-range poultry (n = 192)

Parasite group	Parasite species	Overall prevalence	No. positive (%)		χ^2 (p-value)	No. positive (%)		χ^2 (p-value)
			Female	Male		Young	Adult	
Nematodes	<i>Ascaridia galli</i> (n=105)	21.25%	68 (35.41%)	37 (19.27%)	0.077 (p=0.782)	89 (46.35%)	16 (8.34%)	0.026 (p=0.871)
	<i>Heterakis gallinarum</i> (n=61)	12.35%	45 (23.43%)	16 (8.33%)	2.630 (p=0.105)	55 (28.64%)	6 (3.12%)	2.273 (p=0.132)
	<i>Capillaria</i> spp. (n=24)	4.86%	15 (7.81%)	9 (4.68%)	9.955 (p=0.001)*	17 (8.85%)	7 (3.64%)	3.816 (p=0.05)*
Cestodes	<i>Raillietina echinobothrida</i> (n=104)	21.05%	75 (39.06%)	29 (15.10%)	5.017 (p=0.025)*	92 (47.92%)	12 (6.25%)	1.878 (p=0.171)
	<i>Raillietina</i> sp (n=8)	1.62%	6 (3.12%)	2 (1.04%)	0.325 (p=0.568)	5 (2.60%)	3 (1.56%)	3.030 (p=0.082)

* = significant difference



Figure 1. Infestation of free-range chicken by *Ascarida galli* (A) and *Raillietina* sp (B)



Figure 2. Anterior extremity of *Ascarida galli*



Figure 3. Infestation by *Heterakis gallinarum* in a free-range chicken

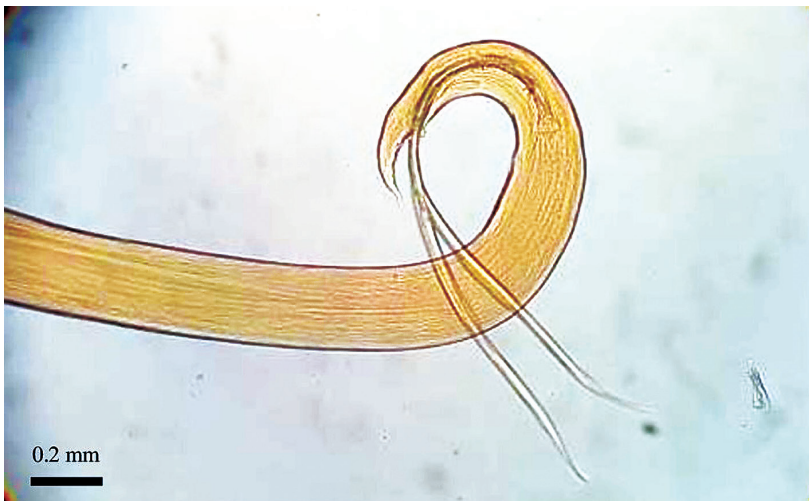


Figure 4. Posterior extremity of a male of *Heterakis gallinarum*



Figure 5. Eggs of *Capillaria* spp

There was a significant prevalence of *Capillaria* spp infections in females (7.81%) ($\chi^2 = 9.955$; $p = 0,001$) and young birds (8.85% ($\chi^2 = 3.816$; $p = 0.05$). Similarly, the presence of *Raillietina echinobothrida* showed a significant association with females ($\chi^2 = 5.017$; $p = 0.025$).

DISCUSSION

This study revealed a relatively low overall prevalence of helminth infections (38.86%) (Total of examined birds: 494), compared to the results of Ben Slimane (2016) (100%), who was interested in thirty animals collected from three regions of Tunisia (Siliana, Boumerdes, and Ghomrasen). This difference could be due to the easier accessibility of the farmers located near the National Veterinary Medicine School, and, consequently, the possibility of veterinary assistance compared to other regions. Our findings were also lower than the reports of many authors in different regions studied worldwide (Table 4).

Table 4. Comparative study of overall prevalence of gastrointestinal helminthes infestation in free-range poultry

Authors	Region of study	Total sample size	Overall prevalence (n = 192)	Nematodes (n = 80)	Cestodes (n = 40)	Mixed infestation (n = 72)
Our study	Northeast of Tunisia	494	38.86%	16.19%	8.09%	15.57%
Naphade and Chaudhari (2013)	Marathwada Region, India	345	84.05%	40%	26.13%	17.97%
Yousfi et al. (2013)	Northwestern, Algeria	124	100%	93.86%	95.61%	90.27%
Mekibib et al. (2014)	Southern, Ethiopia	122	77.80%	67.5%	29.20%	-
Naphade (2014)	Maharashtra state India	317	75.40%	35.96%	22.71%	16.71%
Ben Slimane (2016)	Tunisia: Siliana (north-west); Boumerdas (coastal east); Ghomrasen (south)	30	100%	100%	33.33%	86.66%
Javaregowda et al. (2016)	Shimoga, India	250	73.2%	28.96%	51.36 %	19.67%
Berhe et al. (2019)	Tigray Region, Ethiopia	548	90.98%	84.4%	89%	87.68%
Wuthijaree et al. (2019)	Northern Thailand	211	73.9%	72.5%	27.7%	-

The disparity in these results might be due to a variety of factors, including geographical locations, sample size, study methods, climatic conditions, management practices in poultry flocks, and helminth control measures employed.

Gastrointestinal helminth infections were more frequently observed in young birds under 18 weeks of age (32.80%) compared to adults (> 18 weeks) (6.07%) ($\chi^2 = 12.304$; $p < 0.05$). Females (25.50%) were more frequently infected than males (13.36%) ($\chi^2 = 2.389$; $p > 0.05$). The current findings correspond with the reports of Sarba et al. (2019) regarding the age of animals in different zones of Ethiopia. However, these authors mentioned a higher prevalence in males than females.

The higher prevalence of parasitic infection during rainy and humid seasons is in line with the observations of Mukaratirwa and Hove (2009) in Zimbabwe. In fact, climatic conditions can influence the abundance and the diversity of parasite species.

Among 192 infected animals, 41.67% were found positive for nematodes, 20.33% were found positive for cestodes, and the remaining 37.50% had mixed infections of both cestodes and nematodes. Similarly, Tolossa et al. (2009), Katoch et al. (2012), Baboolal et al. (2012), Mekibib et al. (2014), Ben Slimane (2016), and Wuthijaree et al. (2019) observed a higher prevalence of nematodes than cestodes in backyard chickens in Ethiopia, Jammu (India), Trinidad region, Algeria, Tunisia, and Northern Thailand respectively. Indeed, in agreement with Ruff's report (1999), nematodes constitute the most important group of poultry helminths regarding both infection degree and species number and the resulting tissue damage. On the contrary, Puttalakshamma et al. (2008), Hussien et al. (2012), Idika et al. (2016), Javaregowda et al. (2016), and Berhe et al. (2019) reported a higher prevalence of cestodes followed by nematodes, from different regions.

In the present study, no trematodes were identified. The current findings were in line with the reports of Ben Slimane (2016) and Javaregowda et al. (2016). It might be due to the absence or lower occurrence of the intermediate hosts responsible for the transmission of these parasites in the studied regions (Magwisha et al. 2002).

The relatively high prevalence of gastrointestinal helminths and mixed infections in the present study could be related to environmental factors and poor hygienic conditions in the backyard system, which are very favorable for developing parasitic infections. Animals seek food in the soil, which is frequently contaminated with infective stages of parasites and living organisms (insects, mollusks...) serving as intermediate hosts (Ruff 1999; Yousfi et al., 2013).

The higher prevalence of *Ascaridia galli* (21.25%) followed by *Heterakis gallinarum* (12.35%) has also been reported by several authors (Tolossa et al., 2009; Idika et al., 2016; Javaregowda et al., 2016; Sarba et al. 2019) (Table 5). On the contrary, Yousfi et al. (2013), Mekibib et al. (2014), Abdel Aziz (2016), Ben Slimane (2016), Berhe et al. (2019), and Wuthijaree et al. (2019) reported a higher prevalence of *H. gallinarum* than *A. galli* in local chickens in Algeria, Southern Ethiopia, Egypt, Tunisia, Tigray Region, Ethiopia, and Northern Thailand, respectively.

Table 5. Comparative overall prevalence of intestinal helminthes in free-range poultry

Authors	Region of the study	Total sample size	<i>A. galli</i>	<i>H. gal-linarum</i>	<i>Capil-laria sp</i>	<i>Raillietina echinobo-thrida</i>	<i>Railli-etina sp</i>
Our study	Northeast of Tunisia	494	21.25%	12.35%	4.86%	21.05%	1.62%
Nnadi and George (2010)	South-eastern, Nigeria	261	17.2%	12.6%	5.7%	-	5.7%
Hussen et al. (2012)	Eastern Shewa Zone, Ethiopia	124	32%	37.9%	3.2%**	63.7%	-
Abdullah and Mohammed (2013)	Sulaimani, Iraq	65	31%	81%	1.72%	-	55.17%
Yousfi et al. (2013)	Northwestern, Algeria	124	39.47%	78.07%	35.96%***	85.09%	-
Mekibib et al. (2014)	Southern, Ethiopia	122	45.90%	51.60%	13.1%	17.20%	-
Salam (2015)	Kashmir, India	478	35.35%	4.39%	8.78%**	-	-
Ben Slimane (2016)	Tunisia: Siliana (north-west); Boumerdas (coastal east); Ghomrasen (south)	30	53.33%	100%*	-	-	33.33%
Javaregowda et al. (2016)	Shimoga, India	250	62.3 %	22.6 %	-	9.6 %	-
Berhe et al. (2019)	Tigray Region, Ethiopia	548	68.84%	72.46%	51.45%	39.86%	-
Wuthijaree et al. (2019)	Northern Thailand	211	60.2%	70.9%	44.1%	-	-

Heterakis sp.*; *Capillaria anatis*; ****Capillaria caudinflata*

A. galli is one of poultry’s most common and important helminth infections. It causes diarrhea, growth delay, a decrease in body weight and egg drop in laying chickens (Ashenafi and Eshetu 2004). Blood loss due to intestinal mucosa damage, secondary infections, and intestinal obstruction can be observed in severe infections.

Although *H. gallinarum* is generally non-pathogenic, it plays a crucial role as a potential vector of *Histomonas meleagridis*, a highly pathogenic etiologic agent of “Blackhead disease” (histomoniasis) in many avian species (turkeys, chickens, pheasants). Massive infection by *H. gallinarum* in free-range backyard chickens can cause severe debility, morbidity, and mortality in extreme cases (Ashenafi and Eshetu, 2004).

The prevalence of *Capillaria* spp in the current investigation was 4.86%. Our results disagreed with Ben Slimane (2016) and Rama Rao (2018), who did not detect this helminth in studied regions in Tunisia and India, respectively. However, *Capillaria* spp. was identified in several other regions of the world (Abdel Aziz, 2016; Hussen et al., 2012). Recently, a high prevalence of *Capillaria* spp infections was published by Berhe et al. (2019) (51.45%) and Wuthijaree et al. (2019) (44.1%) in backyard poultry flocks in Ethiopia and Northern Thailand, respectively.

The life cycle of *Capillaria* spp can be indirect (*Capillaria caudinflata*, *Capillaria annulata*) or direct (*Capillaria obsignata*) according to the species. Species with a direct life cycle are more frequently detected under intensive farming conditions where constant temperature and humidity are ideal for larval development. However, species with indirect life cycles are particularly abundant in traditional farms, particularly under humid climates (Permin and Hansen, 1998).

The overall prevalence of cestodes in our study (21.66%) was higher than that reported by Hassan et al. (2020) in live-birds market in Nigeria. However, Naphade and Chaudhari (2013) (26.13%), Yousfi et al. (2013) (95.61%), Mekibib et al. (2014) (29.2%), Naphade (2014) (22.71%), Idika et al. (2016) (70.4%), Javaregowda et al. (2016) (51.36 %) and Sarba et al. (2019) (77.8%) showed a higher prevalence. This could be due to the geographic localization, the difference in the season and the climatic conditions, the availability of intermediate hosts, individual host resistance, and ecological parameters.

Among cestodes, *Raillietina echinobothrida* (21.05%) was the most observed, followed by *Raillietina* spp (1.62%), non-identified because of the bad quality of some samples. *Raillietina echinobothrida* infections might be accompanied by growth delay, decreased egg production in layers, diarrhea, intestinal damage, vitamin B deficiency, and mortality in young birds (Ashenafi and Eshetu, 2004).

The current findings correspond with the report of Idika et al. (2016) (64.5%) in local chickens in Nsukka region of southeastern Nigeria. However, this was not in accordance with the observations of Javaregowda et al. (2016) in Shigoma region (India), who reported higher prevalence of *Raillietina tetragona* infections in backyard chickens (77.6%). Similarly, Butt et al. (2014) reported high prevalence of *Cotugnia digonopora* in Pakistan.

Globally, the prevalence of helminth infections did not differ between female and male birds, indicating that helminth species have no natural affinity for either sex of host chicken. The higher prevalence of *Capillaria* spp. and *Raillietina echinobothrida* in female birds ($p < 0.05$) reported in our study can be explained by reduced immune responses and the supply of untreated new female birds to improve egg production. Physiological, morphological, and behavioral differences between females and males could influence the susceptibility to parasite infections. Many helminth surveys have reported no significant differences according to the sex of birds (Hassouni and Belghyti, 2006; Berhe et al., 2019), whereas others have found significant differences in the favor of one or the other sex (Wuthijaree et al., 2019).

Age is also considered a risk factor associated with the occurrence of helminth infections. The high prevalence of different helminth infections reported globally in young animals compared to adults could be attributed to the immature immune system in young birds and the poor acclimatization of young animals to the immediate environment (Ola-Fadunsin et al., 2019). In addition, Tunisian farmers do not use anthelmintic drugs systematically in free-range chickens.

CONCLUSION

This study showed that free-range chickens reared under backyard systems were infected by different helminth species. The relatively high prevalence of gastrointestinal helminth infections might be associated with a considerable decrease in productivity. The most common helminths identified in the current study were *Ascaridia galli* and *Raillietina echinobothrida*. Several factors can influence the prevalence and severity of infections. Age, sex, and season were considered in the current study. In addition, poor management conditions, mixed animal and avian species, and the negligence of anthelmintic application can preserve the infection in the flock. Therefore, the application of strategic control must thus take priority attention in conjunction with good hygienic practices. Finally, studies are needed to elucidate the economic and hygiene impacts of multiple parasitic infections on poultry reared under a backyard system.

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Author's Contribution

K.K. design of the experimental protocol, parasitological investigations, interpretation of the results, writing the manuscript, N.R. sampling, necropsy protocol, R.B.R. sampling, writing the manuscript, N.A. reception and treatment of samples, M.D. parasitological analysis. All authors read and approved the final version of the manuscript.

Competing interest

The authors declare that they have no competing interests.

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