

Identification of the main intestinal helminths of local breed chickens (*Gallus gallus domesticus* Linnaeus, 1758) reared in traditional mode in the Oran region

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Summary

In order to gain a better etiological and epidemiological knowledge of the parasitic diseases of local breed chickens reared in extensive (traditional) mode, a study was carried out in the Oran region during the periods from February 2020 to April 2020. Ten chickens were examined by the helminthologic autopsy method to identify parasitic helminths in the digestive tract. Four species of helminths have been identified: two species of nematodes: *Ascaridia galli* and *Heterakis gallinarum* and two species of cestodes: *Raillietina cesticillus* and *Raillietina tetragona*. Six chickens were carriers of the parasites, for an overall level of infestation of 60 % with an average infestation of 7 parasites per chicken. The rate of parasitism and infestation varied from species to species, *Heterakis gallinarum* being the most dominant species. The estimate of the infestation rate by each group shows a predominance of nematodes with 62 parasites (88.5 %) compared to cestodes (8 parasites) with a significantly higher difference ($P < 0.05$). The results obtained show that chickens of the local *Gallus gallus domesticus* breed in the Oran region are heavily infested by parasites including *Heterakis gallinarum*, the predominant species.

Keywords: Local breed chickens; extensive mode; helminths; digestive tract; epidemiology; Nematodes; Cestodes

Introduction

Almost 90 % of poultry in developing countries are raised under the extensive system. Local poultry genotypes represent 80 to 99 % of the total numbers of poultry populations present in rural areas (Sonaiya et Swan, 2004). The extensive livestock system is essentially based on traditional farming methods that are not very demanding and that are suitable for village and even urban and peri-urban environments in several African and Asian countries. It is a breeding which is left to itself, generally in the hands of women, the average number of each farm is between 15 and 20 subjects, the hens are fed by rye, Criblure, oats, and kitchen scraps. They are raised in freedom and supplement their diet around the

farm (Belaid, 1986). This is the case for the breeding of local poultry in the Oran regions which are located in rural areas. There are usually chickens, roosters, ducks, geese and turkeys. Backyard chicken is the most common type of farming in these rural areas, being generally affordable for poor rural households. This type of farming contributes significantly to the consumption of animal protein in rural communities in the form of meat and eggs (Mahammi, 2015) and therefore contributes to a balanced and nutritious diet (Ahlers *et al.*, 2009; Mouhous *et al.*, 2012). Traditional poultry farming remains marginalized and practiced mainly in small-scale farming by women in rural areas (Moula *et al.*, 2009).

However, when it comes to chicken production, one of the major constraints is sanitary (Chrysostome *et al.*, 1995). Indeed, many

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diseases of various origins (parasitic, bacterial, viral) affect local chickens and constitute a real scourge which slows down the development of this breeding. Among these diseases, chicken digestive parasitism, which is defined by the presence of parasites in different parts of the digestive tract, from the oral cavity to the cloaca, is a fairly common pathology. The main parasitic diseases linked to this parasitism in chickens are Helminthiasis, which are due to the presence and development in the digestive tract of chickens of pathogenic worms belonging to the classes of Nematodes, followed by Cestodes (Bindoula, 1989) and more rarely Trematodes. (Cribb & O'Callaghan, 1992). Acanthocephali are exceptional in chickens (Reid & Mc Dougald, 1978). The reported prevalence of helminth species varied among species and ranged from 30 to 100 % (Shifaw *et al.*, 2021).

The parasitic species of helminths in the digestive tract are very common. They have a significant economic impact and can cause a significant decrease in productivity (growth retardation, reduction in laying). They promote the spread of deadly infectious diseases and reduce the effectiveness of the immune system, which is ultimately depleted in the long term.

The objective of this study is twofold:

- Search and identify endoparasites in the digestive tract after autopsy of local breed chickens reared in traditional extensive mode in the Oran regions;
- Evaluate the rate of infestation of groups of helminths and the prevalence of each species of helminth.

Material and Methods

Presentation of the study area

This study took place in the wilaya of Oran which covers an area of 2144 km² and which stretches along the Mediterranean coast which forms its natural northern limit. It is located in the north-west of Algeria, 432 km west of the capital Algiers. Overall, the study region is characterized by a Mediterranean climate with a relatively cold and rainy winter and a hot and dry summer. The coastal area is characterized by a mild climate and relatively high humidity.

Target population

The target population consists of local breed chickens present in Algeria. This study concerns 10 chickens during the period from February 2020 to April 2020. We have chosen our sample so that the sex ratio is balanced: 5 males and 5 females. The samples were sent to the Parasitology Laboratory affiliated with Oran 1 University, where the various stages of dissection and recovery of digestive tract parasites were carried out.

Laboratory monitoring methods

Stages of dissection

After the post-mortem phase, the groups of parasites collected and their location were mentioned. The technique applied is that of helminthological autopsy. It proceeds by the mechanical collection

of parasites, the principle of which is the same as in ruminants: isolation of the various parts of the digestive tract (from the esophagus to the cloaca) and searches for helminths in the successive portions by simple sedimentation or filtration – sedimentation.

The steps are as follows: the chicken is euthanized by injecting an air bubble into the heart using a syringe. During the post-mortem phase, the digestive tract is completely removed. It is then dissected into portions (esophagus, crop, proventriculus, gizzard, duodenum, jejunum, ileum, caeca, rectum and cloaca) and placed in Petri dishes containing physiological water or tap water. These portions are then opened longitudinally and left in the aqueous medium for 5 min to promote the detachment of the parasites from the mucosa.

Search and harvest of parasites

The various parasites found are collected after examination, under a binocular magnifying glass with a dark background, of the surface of the mucous membrane section by section. In the case of cestodes, their collection should be done carefully so as not to detach the scolex from the strobile. The rinse water is also tested for parasites for free worms and small worms associated with floating mucus. The parasites are collected separately in vials for large parasites or tubes for small parasites containing ethyl alcohol at 70°. These vials or tubes bear a label in which the sample number is mentioned, the date of collection, the number of parasites, the organ examined and the sex of the chicken.

Parasites study

Treatment and assembly of parasites

The worms undergo various treatments leading to their microscopic observation. The protocols of Mc Laughlin (2003), Pritchard & Kruse (1982), and Georgiev *et al.* (1986) are those that we used during the staining, lightening and mounting stages of the parasites.

Nematodes

The nematodes, fixed with 70° ethanol, are thinned for 48 hours in lactophenol, wiped off with filter paper and then mounted between slide and coverslip in polyvinyl lactophenol. The slides are then dried in an oven at 37° C before being observed under a light microscope.

Cestodes

These fixed worms are immersed in acetic acid for 5 to 10 minutes and then washed with water and 80° ethanol: this is the clarification. Then they are stained with hydrochloric carmine for 24 hours and rinsed with 70° ethanol for 5 to 10 minutes. Differentiation involves immersing the worms in hydrochloric alcohol for 12 hours to remove excess dye. After successively passing through 80° and 95° ethanol for 30 minutes and then 100° for 1 hour for dehydration, the worms are again thinned in toluene for 2 minutes. They are finally mounted between slide and coverslip in Balsam

of Canada and then stored in an oven at 37°C for a week before being observed under a light microscope.

Identification of parasites

The determination of the parasites is made from the study of morpho-anatomical characters and on the basis of the identification keys of helminths established by Euzéby (1961, 1963, 1966).

Statistical analysis

Epidemiological parameters such as the prevalence and distribution of the species listed were determined and analyzed by the XL stat software. 3.1. 2012. Prevalence was determined with a 95 % confidence interval. The risk of error α is set at 5 % which means that when p is less than 0.05, the observed difference is considered significant. For the processing of the photos we used "Leica Application Suite (LAS)" and "Snap Measure v1.7 for Adobe Illustrator 10-CS3".

Ethical Approval and/or Informed Consent

Animal-related research has complied with all national regulations and institutional policies relevant to the care and use of animals as approved by the ethics committee of the University of Oran 1 under reference 02 / CE / UO1 / 2021

Results

The parasitic species collected and their locations

Descriptive and systematic study

The study carried out on local breed chickens reared in extensive mode in the Oran region revealed the presence of four species belonging to different taxa (Table 1).

Nematodes

According to the Euzéby determination key (1961, 1963), we have linked 2 species of nematodes belonging to the Heterakidae family. The identification of these species was made from morpho-anatomical characters.

Ascaridia galli (Schrank, 1788)

Synonym: *Ascaridia lineata* (Schneider, 1866)(Fig.1)

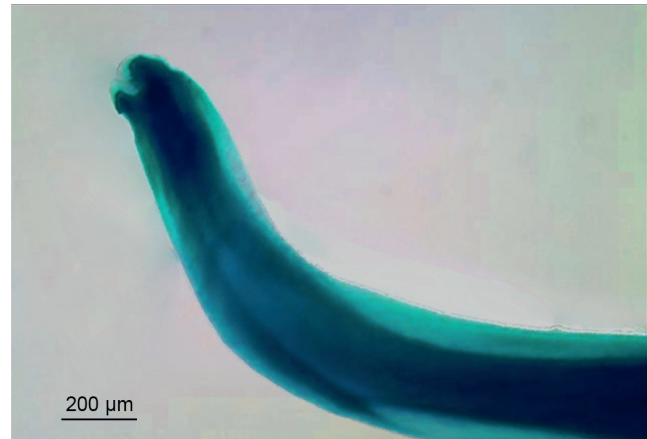


Fig. 1. *Ascaridia galli* (Schrank, 1788), Anterior part, 5 % eosin stain, $\times 40$. The body of this worm is semi-transparent, creamy white, and cylindrical. The anterior end is characterized by a mouth, which is surrounded by three large trilobed lips. The body is completely covered with a transversely striated cuticle. Females are considerably longer and more robust. Males are relatively shorter and smaller.

Description: This worm is the largest nematode in birds. The body is semi-transparent, creamy white and cylindrical. The anterior end is characterized by a mouth, which is surrounded by three large trilobed lips. The body is completely covered with a thick protein structure called the cuticle. The cuticle is striated transversely over the entire length of the body and the cuticular wings are poorly developed. Two conspicuous papillae are located on the dorsal lip and one on each of the subventral lips. These papillae are the sensory organs of the nematode. Females are considerably longer and more robust, measuring 72 to 112 mm in length, with a vulvar opening in the middle part of the body and the anus at the posterior end of the body. The tail of females is typically blunt and straight. Males are relatively shorter and smaller (measuring 50 – 76 mm in length), with a distinct, pointed, curved tail; males possess a pre-cloacal muscular apparatus. The caudal end is truncated obliquely behind the cloaca and has 2 membranous lateral wings. The spicules are subequal. The eggs found in the feces of infected birds are oval with smooth shells and measure 73 – 92 by 45 – 57 microns (Table 2).

Table 1. The different parasitic species collected and their locations

Species	Location	Number of infested chickens
Nematodes		
<i>Ascaridia galli</i>	Different parts of the small intestine	5
<i>Heterakis gallinarum</i>	Caecum	6
Cestodes		
<i>Raillietina cesticillus</i>	Duodenum and jejunum	2
<i>Raillietina tetragona</i>	Duodenum and jejunum	1

Table 2. Comparative characteristics of *Ascaridia galli*

Source	Present study		Euzéby (1963)		Yousfi (2012)		Laouel (2017)	
	Male	Female	Male	Female	Male	Female	Male	Female
Gender								
Length (mm)	21 – 70	40 – 90	50 – 70	80 – 100	17.6 – 71	22.45 – 102	20.83	74
Width (mm)	0.5 – 1.02	0.5 – 1.5	0.60	1.5	0.41 – 1.22	0.46 – 1.5	0.71	0.78
Spicule (mm)	1 – 2.5	/	4	/	0.60 – 2.41	/	0.225	/

Heterakis gallinarum (Schrank, 1788)

Synonym: *H. gallinae* (Gmelin, 1790); *H. papillosa* (Railliet, 1885); *H. vesicularis* (Madsen, 1950). (Fig. 2)

Description: This parasite is 6 to 11 mm in length and 0.25 to 0.34 mm in width. It has two lateral cuticular wings that span the full extent of its body. The mouth is surrounded by three very distinct lips, the stoma is reduced and vestibular, the esophagus is equipped with a posterior bulb enclosing a valve apparatus. The posterior end of the male is tapering and straight, with the presence of two well-developed wings laterally supported by twelve pairs of papillae. The pre-cloacal suction cup is circular with a chitinous mount with a diameter of 0.07 to 0.08 mm. There is no gubernaculum. The spicules are unequal and dissimilar, a slender, long and wingless 1.5 – 2.2 mm long; the other short, 0.5 to 1.1mm wide. The female is 7 to 13 mm in length and 0.30 to 0.40 mm in width, the caudal end is thin and slightly curved in its distal part. The vulva is located slightly behind the middle of the body (Table 3).

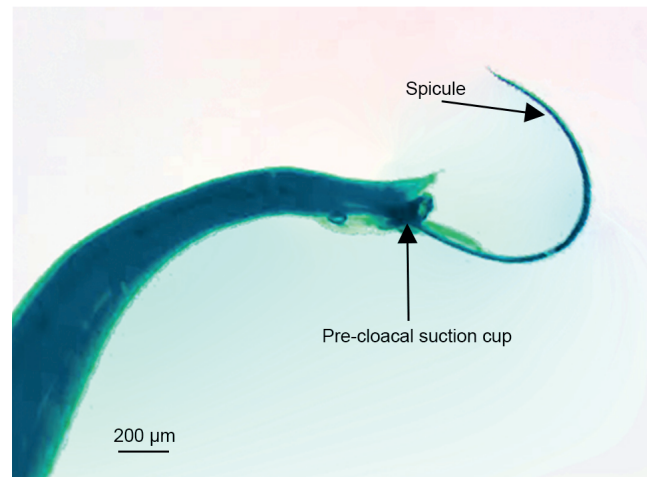


Fig. 2. *Heterakis gallinarum* (Schrank, 1788), 5 % eosin stain, × 40. The pre-cloacal suction cup is circular with a chitinous mount with a diameter of 0.07 to 0.08 mm. There is no gubernaculum. The spicules are unequal and dissimilar, a slender, long and wingless 1.5 – 2.2 mm long; the other short, 0.5 to 1.1mm wide. The caudal end is thin and slightly curved in its distal part.

Table 3. Comparative characteristics of *Heterakis gallinarum*.

Source	Present study		Euzéby (1963)		Yousfi (2012)		Laouel (2017)	
	Male	Female	Male	Female	Male	Female	Male	Female
Gender								
Length (mm)	6 – 11	7 – 13		7.12	5.82 – 10.42	11.25 – 12	6.035	6.67
Width (mm)	0.25 – 0.35	0.30 – 0.40		0.30 – 0.35	0.21 – 0.34		0.35	0.387
Spicule (mm)	1.5 – 2			2 – 2.17	1.33 – 2.12		1.75-2	
				0.70 – 1	0.56 – 0.76			

Cestodes

According to the key for determining the families of parasitic cestodes in chickens (Euzéby, 1963), the two specimens belong to Davainéidae and in relation to the diagnostic criteria, they correspond to the following species:

Raillietina cesticillus (Molin, 1858)

Synonym: *Raillietina mutabilis* (Fig. 3)

Description: It's a little tapeworm. Sometimes called broad-headed

tapeworm. A cosmopolitan parasite widespread in domestic and wild galliformes. It is about 15 cm in length and 1.5 – 3 mm in width. It is whitish in color, very elongated, flattened dorsoventrally and completely covered with a seed coat. The body is made up of the region of the head called the "scolex", an unsegmented "neck" and a very segmented body proper called a strobila. The strobila is made up of a chain of ribbon-shaped proglottids. The scolex bears an apical roundedness, the rostellum, surrounded by four suckers. Unlike other *Raillietina* species, it is unusually large, the rostellum is very prominent and protruding, and the suckers are

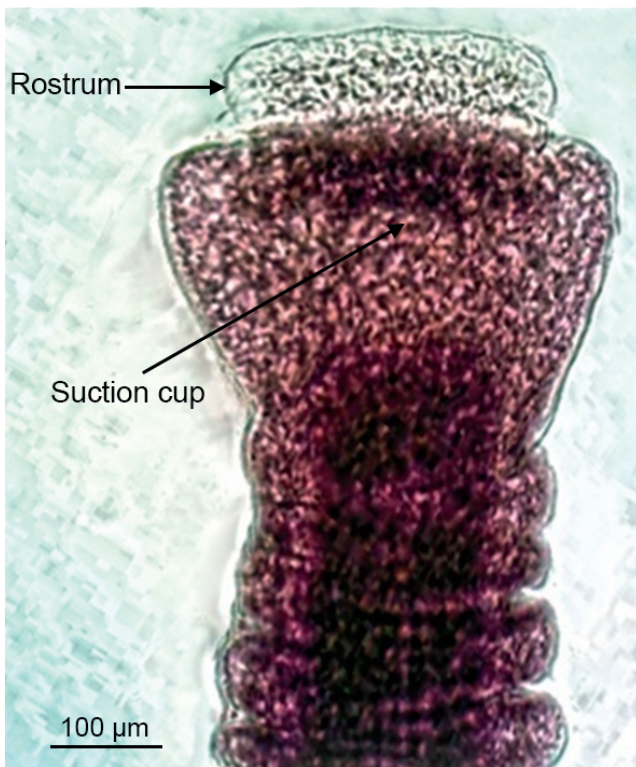


Fig. 3. *Raillietina cesticillus* (Molin, 1858), hematoxylin stain, × 40. The body consists of the 'scolex', an unsegmented 'neck' and a highly segmented body called a strobila made up of a chain of ribbon-shaped proglottids. The scolex bears an apical rounded, the rostellum prominent and protruding, surrounded by four poorly developed suckers completely devoid of thorns.

small. In addition, the rostellar hooks are arranged in two rows. A significant diagnostic feature is an unusually large hook, which can be up to 500. Cupping is poorly developed and completely devoid of special devices or thorns. The scolex is ~ 134 μm in diameter and the hooks are 7 – 10 μm in length.

Raillietina tetragona (Molin, 1858)
 Synonym: *Taenia tetragona* (Molin, 1858) (Fig.4)

Description: this worm is 25 cm in length and 1 to 3 mm in width. It bears a tetragonal scolex 80 to 270 μm in diameter. The latter provided with a rostrum armed with a hundred hooks arranged in a single crown and 4 suction cups 70 to 100 μm in diameter, lined with 8 to 10 rows of small thorns. The genital pores are unilateral and open in the anterior third of the edge of the segments. The ovigerous segments bear oviferous capsules and contain 6 to 12 eggs 25 to 45 μm in diameter.

Study of parasitism
Global infestation rate

The study carried out in the Oran region revealed 6 chickens parasitized out of 10 chickens studied by at least one species of hel-

minth, for an infestation rate of 60 %. We collected, during a period of 3 months, 70 helminths with an average intensity of infestation of 7 parasites per chicken. The estimate of the infestation rate showed a predominance of nematodes with 62 parasites (88.5 %) compared to cestodes (8 parasites i.e. a rate of 11.5 %) with a significantly higher difference ($P < 0.05$). (Fig. 5)

Prevalence of the various helminth species identified

The estimation of the prevalence among the identified helminths revealed high prevalences among the nematodes 60 % and 50 % for *Heterakis gallinarum* and *Ascaridia galli* respectively and low prevalences in cestodes (20 % for *Raillietina cesticillus* and 10 % for *Raillietina tetragona*). (Fig. 6)

Discussion

The present study made it possible to identify within our sample composed of 10 local breed chickens (*Gallus gallus domesticus*) two species of nematodes and two species of cestodes. The prevalence of parasites of the gastrointestinal tract observed in domestic chickens in this study ranged from 10 to 20 % for cestodes and 50 to 60 % for nematodes. These prevalences are much lower than the 90 % recorded by Fabiyi (1972), 92 % by Gadzama

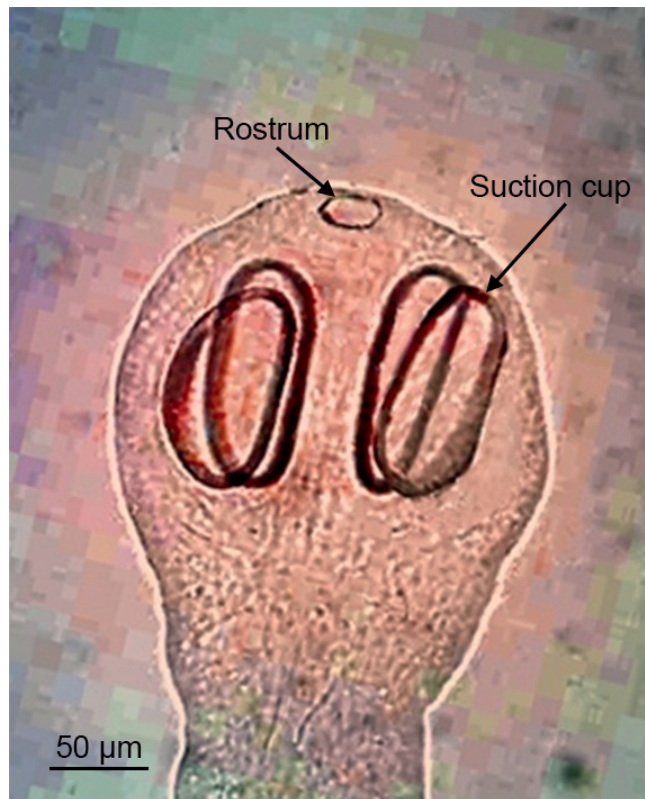


Fig. 4. *Raillietina (Raillietina) tetragona* (Molin, 1858), hematoxylin stain, × 40. This worm has a tetragonal scolex with a rostrum armed with a hundred hooks arranged in a single crown and 4 suckers lined with 8 to 10 rows of small thorns.

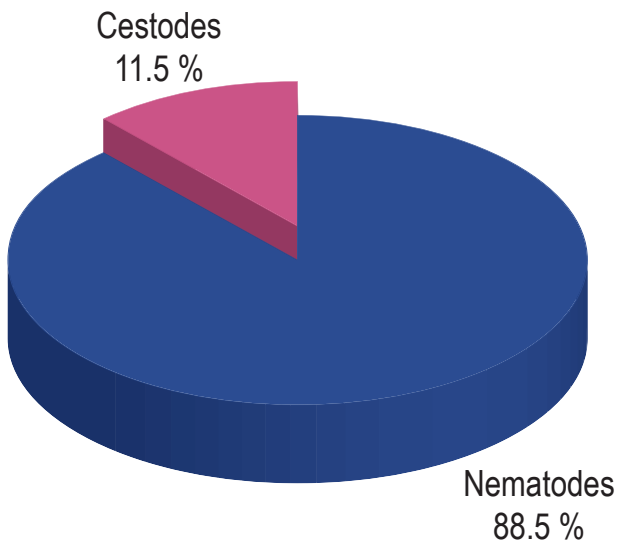


Fig. 5. Infestation rate by identified helminths. The difference between nematodes and cestodes is very significant ($p < 0.05$).

and Strivastava (1986), 100 % by Okon and Enyenihi (1980) and 95.2 % by Fatihu *et al.* (1991). This decrease in overall prevalence would be linked to the general improvement in health habits, which makes the environment less favorable to parasites and their intermediate hosts.

According to Bush *et al.* (1997), species with a prevalence < 30 % are considered as rare species, those with a prevalence > 30 % as common; those with a prevalence > 50 % as the most widespread. According to the values obtained (Fig. 6), *Heterakis gallinarum* (60 %) is the most widespread species followed by *Ascaridia galli* (50 %). The other species can be considered rare, in particular *Raillietina tetragona* (10 %).

The dominance of *Heterakis gallinarum* is reported in several works. The total prevalence of gastrointestinal parasites in indigenous chicken in Kashmir has been estimated at 40.14 % (Raza *et al.*, 2016). In particular, 2 species of nematodes were recovered during the study include *Ascaridia galli* and *Heterakis gallinarum* with a prevalence of (39.53 %) and (41.86 %) respectively (Raza *et al.*, 2016). Shifaw *et al.*, (2021) and according to a meta-analysis instead showed a high prevalence in favor of *Ascaridia galli* (35.9 %), followed by *Heterakis gallinarum* (28.5 %). Likewise, Zada *et al.* (2015) showed a relatively low overall prevalence rate of *A. galli* by about 21.44 %, however *A. galli* was the most common and prevalent nematode in chickens in Mardan district of Pakistan. The study also showed that *A. galli* is a common health problem in free-range chickens and poultry (Zada *et al.*, 2015).

The study carried out by Yousfi (2012) in the Oran region on local chicken also showed a massive infestation of *Heterakis gallinarum* and *Ascaridia galli* in the digestive tract.

The high rate of infection with *Heterakis gallinarum* and *Ascaridia galli* in this study may be influenced by many factors such as the accumulation of infective stages of larvae or eggs in the environ-

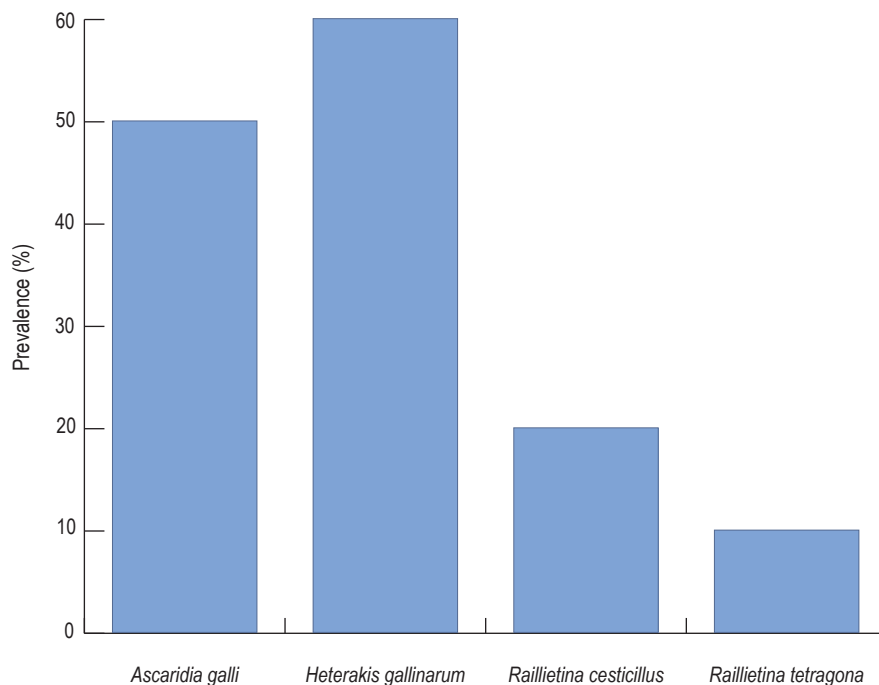


Fig. 6. Prevalence of identified helminth. Among the identified species, *H. gallinarum* represents the most widespread nematode with a rate of 60% followed by *A. galli*. The cestodes *R. cesticillus* and much more *R. tetragona* were found at relatively low rates.

ment, the presence of intermediate hosts, the individual susceptibility of the final host (Magwisha *et al.*, 2002), and the climatic conditions such as Temperature and humidity which promotes larval development and facilitates transmission (Kennedy, 1975; Audu *et al.*, 2004; Magwisha *et al.*, 2002; Dube *et al.*, 2010; Ola-Fadunsin *et al.*, 2019).

According to Andres *et al.* (1998), the establishment of *Ascaridia galli* in the gut is influenced by many factors such as the age of the chicken, the age of the infected eggs, the sex of the chickens and the diet of the host. Infection with this parasite leads to weight loss in chickens, which correlates with an increased parasite load. In general, impacts associated with nematode infections include reduced health, welfare and production performance due to reduced feed conversion ratio, reduced growth rates or weight loss, reduced egg production and egg quality, intestinal damage, and in severe cases, death (Phiri *et al.*, 2007; Sreedevi *et al.*, 2016; Rufai & Jato, 2017). Nematode infections can have direct adverse effects on the host, inducing the breakdown of the gastrointestinal barrier, but indirect damage can also occur via increased susceptibility to secondary infectious diseases (Dube *et al.*, 2010; Sharma *et al.*, 2019) and reduced host immune response (Pleidrup *et al.*, 2014; Dalgaard *et al.*, 2015).

Otherwise, Pinckney *et al.* (2008) indicate that the parasite *Heterakis gallinarum* is not pathogenic in chickens, but can be used for transmission of *Histomonas meleagridis*, a protozoan that can cause severe liver damage in turkeys.

Infection by three species was found in 2 chickens, ie a rate of 20 %. Single infections accounted for 10 % of the total infection, while double infections accounted for 30 %.

The most common type of double infection was roundworms and *Heterakis gallinarum*, which accounted for 70 % of double infections. This result is in agreement with those of Permin *et al.*, 1997; Abubakar and Garba 2000; Luka and Ndams 2007. The diversification of parasitic fauna in the digestive tract of local chickens in the Oran region seems to be linked to the extensive farming method. This allows the chickens to feed on food soiled with helminth eggs and also by insects which act as intermediate hosts.

The present study also showed the presence of cestodes in the digestive tract of local domestic chickens. We found that the prevalence of *Raillietina cesticillus* is very low (20 %) compared to that reported in the work of Hanan *et al.* (2005) in Sudan. Another study in Kashmir showed that infestation of chicken with *Raillietina cesticillus* was estimated at 22.50 % (Javid & Syed, 2013). Concerning *Raillietina tetragona*, its prevalence was weakly evaluated in our study at 10 %, at 14 % in Sudan (Somia & Musa, 2002) but relatively high in India (Raza *et al.*, 2016) with a prevalence of 34.88 % and high (65 %) in Kashmir (Javid & Syed, 2013). In free-living birds, the presence of cestodes in the gastrointestinal tract may be due to the continued ingestion of droppings or infected intermediate hosts such as earthworms, grasshoppers, cockroaches and beetles which are easily accessible in poorly managed stocks as suggested by Majaro (1993). Cestode infection in poultry is known

to cause retarded growth, diarrhea, enteritis, haemorrhages and hypovitaminosis B. In addition to these deleterious effects, heavy infections may also lead to mortality in young birds and lowering or loss of egg production in laying chickens (Layers) (Javid & Syed, 2013).

The results obtained show that chickens of the local *Gallus gallus domesticus* breed in the Oran region are heavily infested by parasites including *Heterakis gallinarum*, the predominant species. Other species such as *Ascaridia galli* and *R. tetragona* responsible for economic losses are also present. The high prevalence rate of these parasites could be a silent source of economic loss for this type of traditional farming. The current study indicates that free range chickens are considered as potential reservoirs for nematode and cestode. This study therefore suggests the need to improve extensive farming conditions in terms of habitat, feeding and periodic pest control which will maximize profits and provide healthy birds for human consumption.

Conflict of interest

Authors have no potential conflict of interest pertaining to this submission to Helminthologia.

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