Helminth communities in the alimentary tract of free raised chickens on rainfed and irrigated agrosystems from southwest Spain

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ABSTRACT
The helminth communities of Extremeña Azul breed chickens (Gallus gallus) have been studied from south-western free-ranging farms in Spain. Two farming systems were compared: a rainfed system and an irrigated system. Necropsy was applied to the animals to compare the structure, biodiversity, and evolution of helminth communities as well as to determine the correlation between parasite burden and chicken weight. 12 species of parasites were identified: 7 cestodes (Raillietina echinobothrida, R. tetragona, Skrjabinina cesticillus, Echinolepis carioca, Davainea proglottina, Amoebotaenia cuneata and Choanoctenia infundibulum), and 5 nematodes (Heterakis gallinarum, Ascaridia galli, Baruscapillaria obsignata, Eucoleus annulatus, and Aonchotheca caudinflata). Cestode prevalence was 54% in the rainfed system whereas it was 98% in the irrigated system; nematode prevalence was 100% in both communities. There were 2 core species in the rainfed and 5 in the irrigated system. The rainfed community showed lower species richness and more dominance. In the irrigated system, the helminth community showed higher biodiversity than in the rainfed system community. This could be attributed to the abundance of intermediate hosts due to irrigation. This study provides data on the distribution of chicken helminths in south-western Europe and the influence of the farming system on their biodiversity.

HIGHLIGHTS
- Helminth communities of free-ranging chickens from two different organic agrosystems (irrigated and rainfed) are compared in southern Spain.
- The irrigated system showed the highest prevalence of helminths. Eucoleus annulatus was only observed in this system with a prevalence of 98%.
- This is the first study carried out in Spain about this issue. It may be of interest to poultry organic farmers.

Introduction
Studies on the epidemiology of chicken helminths in Europe decreased significantly in the second half of the 20th century, coinciding with the introduction of industrial poultry farming. Subsequently, the consolidation of organic poultry farming increased the interest in field studies across Europe (Thapa et al. 2015), especially in Northern European countries, which were pioneers in organic production: Denmark (Permin et al. 1999; Hinrichsen 2015); Sweden (Högblad and Jansson 2011); Germany (Kaufmann et al. 2011; Wongrak et al. 2014) and Austria (Grafl et al. 2017; Zloch et al. 2018). In the scarce data available on chicken parasites are collected in the Index Catalogue of Iberian Zooparasites (Cordero del Campillo et al. 1994).

Thus, the breeding system could play a crucial role on the animals derived from the environment, management, feeding modalities, and methods of parasite control. It has been observed that the prevalence of helminthosis is higher in outdoor than indoor poultry systems due to the higher parasitic risk, although it may also be high in deep litter systems (Permin et al. 1999); and in extensive systems rather than in intensive (Rabbi et al. 2006; Matur et al. 2010; Beyene et al. 2014; Fatima et al. 2015). Although in free-range...
organic systems of laying hens an increase in parasite burden has been shown in contrast to conventional systems, this difference was not significant in terms of prevalence (Wuthijaree et al. 2017).

To the authors’ knowledge, there are no studies about the relationship between artificial field irrigation and helminthosis in free-range chickens. Nevertheless, this positive relation has been described in grazing sheep (Uriarte et al. 1985), rodents (Chaisiri et al. 2012), and humans (Fuseini et al. 2009), where helminth diversity was higher in irrigated areas than in non-irrigated.

This experience was proposed with the aim to know and compare the composition and structure of the helminth communities in chickens bred in two different Spanish agrosystems, namely mountain pastures and areas of irrigated crops in fertile plains.

Material and methods

Animals

For this experiment 100, 6-week-old Extremeña Azul breed chickens were used. The animals were divided into two groups (n = 50) to be transferred to the agrosystems under analysis, a mountain pasture and an irrigated crops in a fertile plain respectively. The animals were reared in indoor parks up to 6 weeks of age. Coprological analyses were carried out to ensure that they were free of helminths and with coccidia levels without pathological significance (<5000 ooquists/g faeces) (Zhang et al. 2013).

Agrosystems

Chickens were moved to rainfed and irrigated agrosystems, located in neighbouring provinces in southwestern Spain. The distance between farms is 250 km. The rainfed farm was located in a pasture (39°24′6.47″ N and 5°40′31.42″ W) in a mountainous area, in Garciaz (Cáceres), at 669 metres above sea level (MASL), with average annual temperature and rainfall of 14.5 °C and 510 mm. The irrigated farm (37°50′18.35″ N and 4°54′4.83″ W) was in the Vega del Guadalquivir, in Villarrubia (Córdoba), at 106 MASL, with average annual temperature and rainfall of 17.8 °C and 612 mm.

Both were organic farms and raised chickens for their own consumption in traditional shelters. The chickens from the study were allocated together with these animals. In both farms the animals were free-ranged during the day and sheltered in perches at night. In the rainfed farm, the food was supplemented with wheat and vegetable scraps, whereas in the irrigation farm they were fed with corn stubble and had access to an area of fruit trees.

The average, minimum and maximum temperatures, number of days of rain, and rainfall during the month prior to slaughter and during the period of stay of the chickens on the farm were calculated.

Experimental design

A total number of 100 animals, 6-week-old chickens, were divided into two groups of 50 and allocated for each agrosystem in mid-August. The animals had an adaptation time of 2 months before they were slaughtered. After this adaptation time (October) 10 animals per group were slaughtered each month until the end of the experiment (February). The age of the chickens was 3.5-month-old at the beginning of the slaughter period and 7.5-month-old at the end. One chicken died in the irrigation group during the experiment, so the total of chickens examined was 99.

Just after the slaughter, the trachea, as well as complete digestive tract of each chicken, was stored, identified in plastic bags and frozen until examination in the laboratory. The trachea and the different sections of the digestive tract were processed separately, by identifying and counting the helminths of each section.

Biological indexes and statistical study

The structure of the helminth community was classified according to Bush and Holmes (1986). Thus, a level of prevalence higher than 66.6% was established for core species, between 33.3% and 65.5% for secondary species, and lower than 33.2% for satellite species. Those species with a prevalence lower than 10% were classified as rare.

QPWeb software (Quantitative Parasitology on the web) was used to obtain prevalence, intensity, and abundance values with 95% confidence (95% CI) (Blaker’s method for prevalence and BCa method with 2000 bootstrap replications for both intensity and abundance). The comparison between communities was performed using Fisher’s exact test and Bootstrap t-test with 1000 replications for prevalences and abundances respectively. P values <.05 were considered significant.

Past software (version 3.14) was used to calculate and compare biodiversity between communities (Berger-Parker dominance, Shannon-Wiener index, and Margalef index).
Table 1. Prevalence, intensity, and abundance (expressed as mean values with 95% CI-brackets-) of helmint species found in both rainfed and irrigated agrosystems.

| Helmint                        | Prevalence (Rainfed) | Prevalence (Irrigated) | Mean intensity (Rainfed) | Mean intensity (Irrigated) | Mean abundance (Rainfed) | Mean abundance (Irrigated) |
|--------------------------------|----------------------|------------------------|--------------------------|---------------------------|--------------------------|---------------------------|
| **Cestodes**                   |                      |                        |                          |                           |                          |                           |
| Raillietina echinobothrida     | 40.0 (26.6–54.1)     | 77.6*** (63.5–87.4)    | 21.6 (11.0–36.8)         | 10.6 (7.6–15.6)           | 8.6 (4.2–16.1)           | 8.2 (5.7–12.2)            |
| Raillietina tetragona          | 4.0 (0.7–13.4)       | 85.7*** (72.9–93.5)    | 3.0 (1.0–3.0)            | 23.8* (16.3–38.6)         | 0.1 (0.0–0.4)            | 20.4** (13.7–33.2)        |
| Skrjabinia cesticillus         | 18.0 (9.4–30.7)      | 22.4 (12.6–36.5)       | 2.4 (1.2–5.2)            | 3.6 (1.8–8.5)             | 0.4 (0.2–1.2)            | 0.8 (0.3–2.1)             |
| Echinolepis carioca            | 2.0 (10.3–33.7)      | 2.0**                  | 3.4 (1.8–8.0)            | 3.0                       | 0.7 (0.3–2.0)            | 0.1                       |
| Choanoetaenia infundibulum     | –                    | 8.2 (2.8–18.9)         | –                        | 2.0 (1.0–3.0)             | –                        | 0.2 (0.0–0.6)             |
| Davainea progolitina           | –                    | 36.7*** (24.1–51.0)    | –                        | 11.1 (9.7–12.8)           | –                        | 4.1 (2.6–5.8)             |
| Amoebotaenia cuneata           | –                    | 18.4** (9.6–31.3)      | –                        | 1.7 (1.2–2.0)             | –                        | 0.3* (0.1–0.6)            |
| Nematodes                      |                      |                        |                          |                           |                          |                           |
| Baruscapillaria obsignata      | 2.0 (0.1–10.3)       | –                      | 1.0                      | –                         | 11.5 (9.5–13.6)          | –                         |
| Aonchotheca caudinflata        | –                    | 67.3** (53.1–79.4)     | –                        | 9.6 (6.2–15.3)            | –                        | 6.5 (4.1–11.0)            |
| Eucoleus annulatus             | –                    | 98.0*** (93.4–100.0)   | –                        | 45.7 (36.6–58.2)          | –                        | 45.7** (37.3–58.8)        |
| Heterakis gallinarum           | 98.0 (89.7–99.9)     | 98.0 (89.5–99.9)       | 11.7 (9.8–13.8)          | 68.5*** (54.0–86.9)       | 10.7 (8.2–14.4)          | 67.1** (51.2–85.1)        |
| Ascaridia galli                | 94.0 (83.6–98.3)     | 49.0*** (34.8–63.5)    | 11.4 (8.7–15.1)          | 4.3** (3.1–6.1)           | 0.0 (0.0–0.1)            | 2.1** (1.3–3.3)           |

*p < .05, **p < .01, ***p < .001. = unable to calculate data.

Results and discussion

Parasitological study. Helmint prevalence, intensity, and abundance

Nowadays, there are few studies about the epidemiology of helmint communities in chickens, since the rise of industrial poultry farming has caused a decrease in traditional poultry systems. In the last years, the growth of organic farming has also spread to poultry, increasing the interest in helmint communities and their epidemiology, particularly in Northern Europe (Permin et al. 1999; Höglund and Jansson 2011; Kaufmann et al. 2011; Wongrak et al. 2014; Hinrichsen 2015; Thapa et al. 2015; Grafl et al. 2017; Zloch et al. 2018). In Southern Europe, there is only one study performed in Italy (Wuthijaree et al. 2017). There are no studies carried out in Spain to date, so this is the first one.

In general, 9173 helmints were isolated during this experience. From the total of parasites recovered, 1603 (17.48%) were observed in the rainfed and 7560 (82.42%) in the irrigated system. This finding suggests higher diversity and richness of helmints in the irrigated system. Although there are no previous studies about the association between irrigated areas and poultry helmintiasi, the association suggested in this study is in concordance with similar studies reported by other authors in grazing sheep (Uriarte et al. 1985), rodents (Chaisiri et al. 2012), and humans (Fuseini et al. 2009) where helmint diversity was higher in irrigated than in non-irrigated areas.

In relation to the taxonomy of helmints observed, 2160 (23.54%) were Cestodes (494 in rainfed and 1666 in irrigated) and 7013 (76.45%) nematodes (1109 in rainfed and 5904 in irrigated). During the parasitological examination, 12 different species of helmints were identified: 7 tapeworms (Raillietina echinobothrida, Raillietina tetragona, Skrjabinia cesticillus, Echinolepis carioca, Choanoetaenia infundibulum, Davainea progolitina and Amoebotaenia cuneata) and 5 nematodes (Baruscapillaria obsignata, Aonchotheca caudinflata, Eucoleus annulatus Heterakis gallinarum and Ascaridia galli).

The number of species identified was 7 (4 cestodes and 3 nematodes) in the rainfed agrosystem and 10 (7 cestodes and 3 nematodes) in the irrigated area. From the total number of species identified during the experience, 6 of them were common to the two agrosystems: R. echinobothrida, R. tetragona, S. cesticillus, E. carioca, A. galli and H. gallinarum. On the other hand, 6 species appeared only in one agrosystem: Baruscapillaria obsignata in the rainfed; C. infundibulum, D. progolitina, A. cuneata, E. annulatus, and A. caudinflata in the irrigated agrosystem). Data of prevalence, intensity, and abundance are shown in Table 1.

Regarding cestodes, we observed a prevalence of 54% in the rainfed system and 98% in the irrigated one. In general terms, these differences in cestode prevalence values were associated with increases in intensity and abundance. These values are higher than those reported in Germany (Kaufmann et al. 2011) as well as in a study carried out jointly in Sweden, Denmark, Netherlands, Belgium, Austria, and Italy (Thapa et al. 2015). In our study, a significant increase in prevalence was observed in the irrigated in comparison with the rainfed system for R. echinobothrida (77.6% and 40% respectively) and R. tetragona (85.7% and 4% respectively). Skrjabinia cesticillus also showed an increase (22.4% vs 18%; but was not significant) in prevalence. The exception was E. carioca whose prevalence was statistically lower in the irrigated (2%) in comparison with the rainfed system (20%). At the same time, certain cestodes were only
observed in the irrigated system: *C. infundibulum* (8.2%), *D. proglottina* (36.7%), and *A. cuneata* (18.4%). These findings suggest an increase of susceptibility in the group of chicken placed in the irrigated system for these parasites, probably in relation to a more adequate environmental condition which would also be connected to the greater presence of intermediate hosts. In the case of *D. proglottina* and *C. infundibulum* the increase in prevalence was associated with peaks of the presence of these parasites in the samples analysed during November and December since cestodes are helminth parasites whose dissemination elements (gravid proglottid and eggs) are extremely sensitive to dehydration and need optimal levels of humidity together with the presence of intermediate hosts. Thus, our findings suggest a relationship between the prevalence of parasites and the presence of humidity-induced by irrigation, in line with previous studies carried out by other authors, where a correlation was found between the prevalence of parasites and soil moisture due to the rain (Fotedar and Khateeb 1986; Skallerup et al. 2005; Mungube et al. 2008; Mwale and Masika 2011; Nagwa et al. 2013; Fatima et al. 2015; Hembram et al. 2015). This fact is also in relation to the structure of helminth communities in both agro-systems, where the same species (i.e. *R. echinobothrida* and *R. tetragona*) were considered core species in the irrigated system and secondary and rare species in rainfed respectively.

Regarding nematodes, there were 2 species in common in both agro-systems (*H. gallinarum* and *A. galli*). These helminth species are common parasites in chickens and are usually observed during necropsy or coprological studies (Jansson et al. 2010; Thapa et al. 2015). Prevalence values for *H. gallinarum* were the same in both systems (98%), what suggests the cosmopolitan character of this parasite, as has been previously reported by other authors (Wuthijaree et al. 2017; Cupo and Beckstead 2019), although other studies have reported a large variation in prevalence across Europe for this parasite (Thapa et al. 2015). Moreover, there was a significant decrease of *A. galli* prevalence in the irrigated system (49%) in comparison with the rainfed (94%). In this case, this decrease in the prevalence of *A. galli* was also associated with a significant decrease in intensity and abundance, particularly marked in January. These values are higher than reported by other studies carried out in Sweden (Jansson et al. 2010) but similar to recent studies performed in 8 European countries (Thapa et al. 2015). This decrease in prevalence could be indirectly motivated by soil moisture, favouring the presence of microfungi with ovicidal capacity (Braga et al. 2012). Another hypothesis could be related to longer grazing times, which could be inversely proportional to the burden of *A. galli* (Thapa et al. 2015), as in the irrigated area chickens were free to roost whereas in the rainfed area they were collected from their roost before dusk to protect them from predators. Similar to the case of cestodes, there were some species only observed in one agro-system. In the case of the rainfed system *B. obsignata* was observed, although the prevalence value was discrete (2%). In the irrigated system *A. caudinflata* (67%) and *E. annulatus* (98%) were observed, both with high prevalence values. This fact could be related to the availability of earthworms acting as intermediate hosts (Cram 1936; Allen 1950; Anderson 2000). In several studies performed in Europe, intestinal capillaria appeared with high prevalence in free range/organic systems (Permin et al. 1999; Kaufmann et al. 2011; Wongrak et al., 2014; Wuthijaree et al. 2017), being the most common *A. caudinflata*. However, infections with the oesophageal capillaria *E. annulatus* were not recorded in chickens.

### Table 2. Species structure of the rainfed and irrigated chicken helminth communities.

| Rainfed helminth community | Irrigated helminth community |
|----------------------------|-----------------------------|
| **Core species**            | **Core species**            |
| *Heterakis gallinarum*      | *Heterakis gallinarum*      |
| *Ascarididia galli*         | *Ascarididia galli*         |
| **Prevalence (%)**          | **Prevalence (%)**          |
| 98.0                       | 100.0                       |
| 94.0                       | 98.0                        |
| **Secondary species**       | **Secondary species**       |
| *Raillietina echinobothrida*| *Raillietina echinobothrida*|
| **Prevalence (%)**          | **Prevalence (%)**          |
| 40.0                       | 49.0                        |
| **Satellite species**       | **Satellite species**       |
| *Echinolepis carioca*       | *Echinolepis carioca*       |
| **Prevalence (%)**          | **Prevalence (%)**          |
| 20.0                       | 22.4                        |
| *Skrababinia cesticillus*   | *Skrababinia cesticillus*   |
| **Prevalence (%)**          | **Prevalence (%)**          |
| 18.0                       | 18.4                        |
| **Rare species**            | **Rare species**            |
| *Raillietina tetragona*     | *Choamotaenia infundibulum*|
| **Prevalence (%)**          | **Prevalence (%)**          |
| 4.0                        | 8.2                         |
| *Baruscapillaria obsignata* | *Echinolepis carioca*       |
| **Prevalence (%)**          | **Prevalence (%)**          |
| 2.0                        | 2.0                         |
to date. Although *E. annulatus* has been described as a cosmopolitan parasite by some authors (Permin and Hansen, 1998) it has been rarely observed in chickens in Europe (Kurt and Acici, 2008). Therefore, this study describes parasitism in chickens by *E. annulatus* for the first time.

**Helminth community structure**

The structure of the helminth community in rainfed and irrigated agrosystems is showed in Table 2. As commented in the material and methods section, according to prevalence values, the species were classified as core, secondary, satellites, and rare. There were differences in the composition of the helminth community in both agrosystems. Only two species occupied the same position and classification in rainfed and irrigated agrosystems: *H. gallinarum* as core specie and *S. cesticillus* as satellite specie.

**Biodiversity**

The biological biodiversity of helminths from both agrosystems was estimated and the parameters considered are shown in Table 3. Helminth community from the irrigated agrosystem showed an increase in both, the number of taxa (S) and total abundance. According to the Margalef index both agrosystems were classified as poor specific richness areas (only 12 helminth species were found in all chicken analysed). This is in concordance with the Shannon index, the values of which for both agrosystems were indicative of low diversity areas. Despite these data, the irrigated area showed higher specific richness and biodiversity than the rainfed. Consequently, when the Berger-Parker index of dominance was evaluated, the irrigated agrosystem also showed a lower value in comparison with the rainfed agrosystem, suggesting lower dominance in the irrigated system because of high diversity.

Finally, the presence of certain nematodes species only in the irrigated system (*A. caudinflata* and *E. annulatus*) could be related to the availability of earthworms which act as intermediate hosts (Cram 1936; Allen 1950; Anderson 2000). In several studies performed in Europe, intestinal capillaria appeared with high prevalence in a free range/organic systems (Permin et al. 1999; Kaufmann et al. 2011; Wongrak et al., 2014; Wuthijaree et al. 2017), being the most common *A. caudinflata*. However, infections with the oesophageal capillaria *E. annulatus* were not recorded in chickens before. This study describes parasitism in chickens by *E. annulatus* for the first time.

**Conclusions**

In summary, from the two systems compared, the irrigated system has shown more prevalence of helminths in general (cestodes and nematodes). Although none of the systems showed higher diversity (only 12 different species in total), the irrigated system has shown higher species richness and biodiversity.

From the 6 species common to both systems, 2 cestodes (*R. echinobotrida* and *R. tetragona*) showed higher prevalence values in the irrigated system. On the other hand, only one nematode specie (*A. galli*) showed a significant decrease in prevalence in this system. There were species observed only in the irrigated system because of the presence of moisture in the soil derived from irrigation.

This is the first study carried out in Southern Europe and particularly in Southwest Spain about helminth communities from the alimentary tract on free-raised chickens. More studies are needed to deepen the knowledge about helminthic communities in this kind of farming.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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The data that support the findings of this study are available from the corresponding author (F.J. Martínez-Moreno), upon reasonable request.

**Data availability statement**

Data available on request from the authors.

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