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Insects Associated with Poultry Litter on a Poultry Farm in Pinar del Río, Cuba

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ABSTRACT

Background: Many of the insects present in poultry litter serve as intermediate hosts for endoparasites that can compromise the welfare of poultry and negatively impact productive performance indicators. **Aim.** To identify the main groups of insects found in poultry litter on a poultry farm and to investigate the presence of helminths. **Methods:** The study was conducted on a White Leghorn layer farm located in western Cuba. One kilogram of poultry litter was collected from each shed to quantify and identify the arthropods. Identification was carried out at the Provincial Animal Health Laboratory in Pinar del Río. The proportion of each arthropod species in the sample was determined, along with the level of environmental infestation based on the total number of *Alphitobius diaperinus*. The statistical analysis was performed using the COMPAPROP 1.0 software. **Results:** A total of 617 arthropods were collected, represented by three species of coleopterans: *A. diaperinus*, *Dermestes ater*, *Tenebrio* sp. and one specimen of earwig (*Forficula auricularia*). The environmental infestation level of *Alphitobius diaperinus* on the farm was classified as moderate, with a total of 205 beetles recorded. *Forficula auricularia* stood out as the most representative species, accounting for 36% of the sample. **Conclusions:** The study revealed a dominance of *Forficula auricularia*, while *Alphitobius diaperinus* exhibited a moderate level of infestation. The exclusive identification of the endoparasites *Subulura suctorica* and cysticercoids in *Alphitobius diaperinus* was noteworthy. This finding is of particular relevance to poultry biosecurity in Cuba, as it underscores the need to strengthen sanitary controls

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against this common pest in poultry facilities, in order to prevent health risks and safeguard animal welfare.

Keywords: *Alphitobius diaperinus*, endoparasites, laying hens, Cuba, *Subulura suctoria* (Source: AGROVOC)

INTRODUCTION

The presence of arthropod species poses a challenge to achieving optimal productive efficiency; their emergence is associated with environmental conditions favorable to their development, as well as factors related to the lack of preventive measures (Hernández *et al.*, 2013). Beetles serve as intermediate hosts for more than 50 parasitic helminths, including tapeworms (Cestoda), flukes (Trematoda), roundworms (Nematoda), and thorny-headed worms (Acanthocephala). These parasites primarily infect non-human hosts. They can be toxic to domestic animals when ingested, act as mechanical vectors of pathogenic agents, and cause structural damage to poultry facilities. On the other hand, they can also be beneficial, as they contribute to the recycling of animal waste and act as natural controllers of flies that breed in manure. In poultry production units, coleopterans inhabit the manure towers beneath caged birds and the litter of floor-raised birds. Most tenebrionids are scavengers that feed on dry or decaying plant material, although some feed on live plants (Rodríguez *et al.*, 2013; Krinsky, 2019).

The lesser mealworm, *Alphitobius diaperinus* (Panzer, 1797), has a cosmopolitan distribution. Although its origin on the African continent remains uncertain, several authors suggest that it may have originated in the sub-Saharan region (Vaughn *et al.*, 1984; Geden & Hogsette, 1994). It is a species adapted to warm and humid climates, found in a wide variety of habitats (Sparagano *et al.*, 2018), making its spread in tropical countries such as Cuba particularly favorable. Its role as a vector represents the most significant risk. Throughout production cycles, this beetle contributes to the persistence of contamination and the prevalence of various diseases caused by bacteria (*Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli*, *Clostridium* spp.) and viruses (Marek's disease, avian influenza, and turkey coronavirus) (Hazeleger *et al.*, 2008; Rodríguez, 2023; Sammarco *et al.*, 2023). On the other hand, the beetle *Dermestes ater* (DeGeer, 1774) is responsible for the transmission of cestodes such as: *Choanotaenia infundibulum* and *Raillietina laticanalis* in birds (Avancini & Ueta, 1990). Earwigs (Dermaptera) are primarily omnivorous insects with a preference for plant material, but they are also considered generalist predators and effective pest controllers (Cañellas *et al.*, 2005; Romero Sueldo de Escaño & Virla, 2009). Some species serve as intermediate hosts for the nematodes *Cheilospirura hamulosa* (Sánchez, 2010) and *Tropisurus confusus* (Birova *et al.*, 1980).

In Cuba, knowledge about insect populations associated with poultry litter in poultry farms and their role as intermediate hosts of endoparasites is limited, with studies confined to specific provinces over the past ten years, e.g., the work of Gorrín *et al.* (2017) and González (2023) in poultry farms in the province of Artemisa (western region) and the province of Cienfuegos, respectively; whereas Rodríguez and Grillo (2019) investigated only the presence of insects

associated with quail litter in the province of Villa Clara (central region). Considering these precedents, the present study aims to identify the main insect groups present in poultry litter from a layer farm in the province of Pinar del Río, and to investigate the presence of helminths responsible for parasitism.

MATERIALS AND METHODS

Study Area

The field study was conducted at a layer hen farm (*Gallus domesticus*, Linnaeus, 1758) (Aves: Phasianidae) of the White Leghorn breed, located at kilometer 14 on the road to the town of Antonio Briones Montoto, Pinar del Río, and belonging to the Western Cuba Poultry Company (Empresa Avícola del Occidente de Cuba). It is epidemiologically unprotected (76 points), according to the results obtained from the biosecurity assessment for poultry farms under Resolution 76/2015 of CENASA (National Animal Health Center) in the province of Pinar del Río, conducted in 2022. The farm has the capacity to house 56,000 laying hens in eight active poultry houses, arranged in parallel and oriented east–west. Current capacity utilization stands at 99.75% (55,860 laying hens). The laying hens were housed in battery cages at a rate of four birds per cell, under uniform conditions of housing, management, and feeding, according to Madrazo *et al.* (2020).

Poultry litter sampling

Poultry litter sampling was carried out to quantify and identify arthropods from the farm with the highest level of vulnerability. In each poultry house, four individual subsamples of 250 g of poultry litter were collected—from the beginning, center ($\times 2$), and end of the manure pit—until completing one kilogram. In this context, a composite sample was formed and analyzed as a whole. Subsequently, arthropods were extracted from each kilogram of poultry litter using forceps and placed in plastic containers with 15 g of feed composed of concentrate, bread, and portions of excreta, in order to preserve their viability for 24 hours under conditions similar to their natural environment. Species identification was carried out in the Parasitology Area of the Provincial Animal Health Laboratory of Pinar del Río. Identification of *Tenebrio* beetles was carried out only at the genus level, since most species are scavengers and are not considered relevant vectors or of priority sanitary interest. For this reason, this level of identification was considered sufficient for the purposes of the present study.

Identification of endoparasites in the laboratory

The number of arthropods collected per poultry house was quantified, and the presence of nematode and cestode larvae was investigated. For laboratory identification, the methodology employed was the technique described by Ovies and Jurásek (1970) and Jurásek and Espaine (1971): Immobilization of the beetle using dissection forceps, separation of the head from the rest

of the body, extraction of the internal contents onto a microscope slide by applying caudo-cranial pressure along the beetle's body, addition of two drops of tap water to the sample to prevent dehydration, placement of another slide over the extracted material to observe the entire contents by squash technique, and examination of the material under a light microscope using a 10× objective.

Determination of environmental infestation level by *A. diaperinus*

The level of environmental infestation was determined based on the total number of *A. diaperinus* found in the four poultry houses of the analyzed farm, categorized as follows: more than 300 individuals indicated a highly infested environment; between 100 and 299, a moderately infested environment; and between 25 and 99, an infested environment. Fewer than 25: practically uninfested, according to the criteria referred to by Hosen *et al.* (2004).

Statistical analysis

A comparison of proportions between arthropod species was performed, and Duncan's multiple range test was applied to demonstrate differences among them at a 95% confidence level. The statistical software COMPAPROP 1.0 (Duvergel and Miranda, 2014) was used.

RESULTS AND DISCUSSION

A total of 617 arthropods were collected, represented by three species of coleopterans: *A. diaperinus*, *D. ater*, *Tenebrio* sp. (Coleoptera Linnaeus, 1758); and earwigs *Forficula auricularia* (Dermaptera DeGeer, 1773) associated with poultry litter from *G. gallus domesticus*. The level of environmental infestation by *A. diaperinus* on the farm was classified as moderately infested, since only 205 beetles were counted (41.08%) (Table 1).

Table 1. Arthropods associated with poultry litter in a poultry farm in Pinar del Río province.

Order	Family	Species	Total	Proportion (%)	Significance /±SE
Dermaptera	Forficulidae	<i>F. auricularia</i>	224	36.30 ^a	***/1.74
Coleoptera	Dermestidae	<i>D. ater</i>	70	11.35 ^c	
Coleoptera	Tenebrionidae	<i>A. Diaperinus</i>	205	33.23 ^a	
Coleoptera	Tenebrionidae	<i>Tenebrio</i> sp.	118	19.12 ^b	

***Different superscripts (a, b, c) within the same column indicate statistically significant differences at $p < 0.001$. ±SE: Standard error.

In this study, *F. auricularia* emerged as the most representative species, accounting for 36% of the total, followed by *A. diaperinus* (33%) and *Tenebrio* sp. (19 %). *Dermestes ater* exhibited a significantly lower proportion (11%). Gorrín *et al.* (2017) identified two main insect species

associated with poultry litter on a poultry farm in Artemisa Province, Cuba. *A. diaperinus* was the most represented species in the samples (67%) compared to *F. auricularia* (33%). In a subsequent study conducted in the central region of Cuba, Rodríguez and Grillo (2019) identified nine insect species associated with quail litter on a poultry farm in Villa Clara. The identified coleopterans were *Carcinops pumilio* (Erichson, 1834) (Coleoptera: Histeridae), *Trox insularis* (Chevrolat, 1864) (Coleoptera: Trogidae) and *A. diaperinus*, the latter accounted for 23.34% of the collected species. The order Dermaptera showed a low percentage (5.34%). Di Iorio and Turienzo (2011) reported 144 insect species associated with the habitat of *Gallus* (Aves: Phasianidae) in the Neotropical region, of which 42 belong to the order Coleoptera and 5 to the order Dermaptera. Although *A. diaperinus* is a common species in poultry facilities, it has also been reported in the bedding of guinea pig (*Cavia porcellus* Linnaeus, 1758) farms (Salgado-Moreno *et al.*, 2022).

The role of earwigs as beetle predators should be considered when relating them to the number of coleopterans, since they feed on the larvae of these insects and could potentially serve as a form of biological control (Quintero *et al.*, 2015). In Cuba, it is unknown whether earwigs might be playing a role in the control of other insects in poultry farms, thereby affecting their populations. Its presence and interaction with other arthropods in poultry litter is poorly studied, and there are no recent investigations regarding its role as an intermediate host or vector of pathogens in laying hens. Another factor to consider is the presence of predators or biological agents, as insects may be affected by predation or by diseases caused by parasites, bacteria, fungi, or viruses.

Endoparasites were identified only in *A. diaperinus*. A total of 28 infective third-stage larvae of the nematode *Subulura suctoria* and 7 cysticercoids were quantified. In previous years, *S. suctoria* ranked among the helminths with the highest incidence in the centers of the National Poultry Complex. This nematode develops its biological cycle in an indirect and natural manner, using invertebrates as intermediate hosts, including *A. diaperinus* (Baruš, 1968), which holds greater epizootiological value for *S. suctoria* (Tamayo *et al.*, 1991), and *Labidura riparia* Pallas, 1773 (Dermaptera: Labiduridae). which appears in Baruš (1970) as *Labidura bidens* Oliver, 1791; and as *D. ater* (Sánchez, 2010; Roque, 2015). In 1970, Baruš identified, within an experimental context in Cuba, five new species that serve as intermediate hosts for the nematode *S. suctoria*. These were: *D. ater*, *Opatrinus pullus* Sahlberg, 1823 (Coleoptera: Tenebrionidae), *Pycnoscelus surinamensis* Linnaeus, 1758 (Blattodea: Blaberidae), *Conocephalus brevipennis* Scudder, 1862 (Orthoptera: Tettigoniidae), and *Orphulella scudderi* Bolívar, 1888 (= *Parachloebata scudderi*) (Orthoptera: Acrididae).

Beetles carrying pathogens on their exoskeleton or within their digestive tract can transmit them to chickens when ingested, particularly to young animals, which have been documented to consume hundreds of beetle larvae in a single day (Sammarco *et al.*, 2023). In the gut of *A. diaperinus*, microbial communities can persist for five weeks or more after removal from

contaminated environments, potentially enabling this beetle to transmit pathogens between flocks (Crippen *et al.*, 2022).

Nematodes are generally not a common issue in laying hens housed in conventional cages, but are more frequently found in alternative systems, such as when birds are raised on the floor (Soulsby, 1987). Although the marked affinity of *S. suctorica* larvae for intermediate hosts of the Tenebrionidae family is an ecologically evident fact (Baruš, 1970), this nematode is a mildly pathogenic worm that does not produce overt clinical symptoms. However, when infestation is severe, irritative lesions of the mucosa are observed when it occurs in combination with *Heterakis gallinae*, and clinical signs similar to those described for other parasitic infections may then appear (Sánchez, 2010). *Alphitobius diaperinus* can also be invaded by larvae of the nematode *Hadjelia truncata*, with infection rates of 66.2% under natural conditions and 45.1% under experimental conditions (Alborzi and Rahbar, 2012).

Several species of cestodes can develop the cysticercoid stage in *A. diaperinus*: *Raillietina cesticillus*, *Raillietina magninumida*, *Choanotaenia infundibulum* e *Hymenolepis carioca* (Escobar *et al.*, 2010). In a study of beetles conducted in Egypt, 20% of the *A. diaperinus* examined were infested with cysticercoids of *Hymenolepis diminuta*, found in the hemocoel (Mazen, 2006). Although in that investigation this beetle was found to be free of endoparasites, in another study conducted in Brazil, *D. ater* was found to harbor the cestodes *C. infundibulum* and *R. laticanalis*. In April and May 1988, low infection rates were observed that may not be related to the parasite's natural cycle but rather to changes in management of the poultry facility during those particular months (Avancini and Ueta, 1990).

CONCLUSION

The study demonstrated the dominance of *F. auricularia* on the evaluated poultry farm, while *A. diaperinus* showed a moderate infestation. Notably, endoparasites *S. suctorica* and cysticercoids were found exclusively in *A. diaperinus*, confirming its potential role as a vector of pathogens for poultry. This finding is particularly important for poultry biosecurity in Cuba, as it underscores the need to strengthen sanitary controls on this common pest in poultry facilities to prevent parasitic risks, safeguard animal health, and ensure food safety.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: JPA, MGN, TMR, MCC, data analysis and interpretation: JPA, MGN, JAGG, GGA, writing of the manuscript: JPA, TMR, JAGG, GGA, MCC.

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.