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Original

Clinical Signs and Lesions in Laying Hens with Bacterial and Helminth Coinfections in Western Cuba

Manuel Colas Chavez *, Odalys Correoso Mendoza **, Karel Bartelemy Carmenaty **, Daisy Rodríguez García **, Moraima Aguilera Palmero **, Gabriel Gorrín Armas ***,

*Center for Hematology and Immunology. Center for Poultry Research Havana, Cuba.
**Jesus Menendez Laboratory for Poultry Diagnostic, Center for Poultry Research, Cuba.
***Center for Parasitological and Vector Studies (CEPAVE) (UNLP-CONICET). La Plata, Buenos Aires, Argentina.
Correspondence: olimpia731026@gmail.com

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ABSTRACT

Background: Enteric diseases pose a growing threat to poultry production, particularly due to underdiagnosed coinfections between Enterobacteriaceae and helminths. These coinfections cause economic losses because their clinical signs and lesions are nonspecific, complicating diagnosis. **Aim**: To analyze associations between clinical signs and anatomopathological lesions in laying hens affected by coinfections on three farms in western Cuba. **Methods**: An observational study was conducted on 30 White Leghorn laying hens, 83 weeks old, presenting signs of enteric disease. Diagnosis was established by microbiological isolation of enterobacteria and morphological identification of helminths at necropsy. Chi-square tests and the phi (φ) correlation coefficient were used to evaluate associations between coinfections and clinicopathological findings. **Results**: The most frequent clinical signs were diarrhea (60%) and ruffled feathers (53.3%), while predominant lesions were catarrhal enteritis (70%) and hepatic degeneration (56.7%). The coinfection *Subulura suctoria* + enterobacteria showed a significant association with diarrhea (χ^2=8.17; p=0.004) and a moderate negative correlation. This coinfection was also significantly associated with hepatic degeneration, ovarian degeneration, and ovarian atrophy. Enterobacterial coinfections exhibited moderate correlations and trends approaching significance with catarrhal enteritis and gaseous typhlitis. **Conclusions**: These findings underscore the importance of managing bacterial and parasitic coinfections in laying hens to reduce the incidence of severe lesions that compromise productivity and animal welfare, with an emphasis on One Health. **Keywords**: poultry; Enterobacteriaceae; laying hens; helminths. *Source: AGROVOC*)

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INTRODUCTION

Despite the substantial contributions of the poultry sector to the economy and to the population's livelihoods, it faces numerous challenges, particularly coinfections involving enterobacteria and gastrointestinal helminths. Within the family Enterobacteriaceae, *Salmonella* spp. and *Escherichia coli* exhibit very high levels of antimicrobial resistance to antibiotics, jeopardizing the viability of the poultry industry. Vulnerabilities that compromise biosecurity in poultry facilities can lead to outbreaks of enterobacteriosis, in which *E. coli* prevalence may reach up to 50%. The presence of enterobacteria also acts as a predisposing factor for the emergence and progression of other avian diseases, potentially aggravating their clinicopathological presentation (Villalón *et al*., 2017; Fonseca *et al*., 2018; Peña *et al*., 2019; Domínguez and Pinto, 2020; Aidaros *et al*., 2022).

Helminthiasis generally produces highly nonspecific clinical signs, which often impede presumptive diagnosis. Affected birds exhibit generalized clinical manifestations, reduced productive performance, and in some cases death due to intestinal obstruction and mucosal rupture. Lesions range from catarrhal to hemorrhagic enteritis depending on the severity of the infection. Diagnosis is primarily based on postmortem examination or on the microscopic detection of eggs or parasites (Abd El-Ghany, 2022). Cestode infections that significantly impair poultry productivity are caused by *Choanotenia infundibulum*, *Davainea proglottina*, and *Raillietina* spp., principally *Raillietina cesticillus*. The most common nematode infections are due to *Ascaridia galli*, *Heterakis gallinarum*, *Capillaria* spp., *Strongyloides* spp., and *Syngamus trachea*. Helminths are more prevalent in alternative rearing systems, such as backyard and free-range production (Sánchez 2010; Das *et al*., 2020; Shifaw *et al*., 2021; Jilo *et al*., 2022; Salawu and Emmanuel, 2023).

In Cuba, the cestodes of greatest prevalence and clinical significance are *Choanotenia infundibulum*, *Raillietina cesticillus*, and other *Raillietina* spp. Conversely, nematode infections are primarily associated with *Ascaridia galli*, *Heterakis gallinarum*, and *Subulura suctoria*. These infections occur either singly or in mixed assemblages, sometimes in combination with highly pathogenic species, thereby posing a risk to avian health (Quintana *et al*., 2016; Rodríguez *et al*., 2018; Ferrer *et al*., 2018). Few studies have systematically examined enterobacterial infections in association with helminthiasis; most investigations have focused on links with coccidiosis, avian leukosis, nutritional deficiencies, and mycoses, without thoroughly exploring their relationship to the clinicopathological picture (Pacheco and Carrazana, 2015; Rodríguez *et al*., 2016a; Villalón *et al*., 2017).

Over the past five years, no studies have systematically integrated coinfections by enterobacteria and helminths in laying hens, particularly with respect to their clinical symptomatology and

anatomopathological lesions. This gap limits understanding of the clinicopathological presentation of these diseases within current poultry production systems. Consequently, it is essential to generate updated scientific evidence to characterize these manifestations, thereby contributing to differential diagnosis and to the improvement of sanitary management strategies. In this context, the present study aims to analyze the associations between clinical signs and anatomopathological lesions in laying hens affected by enterobacteria and helminths on farms in western Cuba.

MATERIALS AND METHODS

Study design and study population

An observational, cross-sectional, descriptive study was conducted. A non-probability convenience sampling method was used. The study comprised a total of 30 White Leghorn (*Gallus domesticus*) laying hens, 83 weeks of age, originating from three conventional poultry farms in western Cuba. Birds were selected on the basis of clinical signs consistent with enteric disease and reductions in productive performance indicators (egg production, laying percentage, feed conversion).

Inclusion criteria and diagnosis

Hens presenting clinical signs such as diarrhea, dehydration, depression, weight loss, ruffled plumage, pallor, retraction of the combs and wattles, and emaciation (Figure 1) were included. A positive diagnosis was established by isolation and microbiological identification of enterobacteria from liver, spleen, and intestinal content samples on selective media (Eosin Methylene Blue agar, Nutrient agar, MacConkey agar, and Salmonella-Shigella [SS] agar) and by conventional biochemical testing (ENTERO WELL D-ONE® kit, in accordance with Directive 98/79/EC on *in vitro* diagnostic medical devices C.P.M.). Parasitological diagnosis was performed using helminthological techniques and morphological identification of adult parasites at necropsy, following the morphological keys and descriptions of Soulsby (1982) and Jones and Bray (1994).

Figure 1. White Leghorn layer exhibiting visible signs of depression, comb retraction, and ruffled plumage

Necropsy and anatomopathological evaluation

The hens were euthanized by exsanguination following cervical spinal cord transection. Necropsy was performed according to the methodology described by Colas *et al*. (2010). Each organ sample was macroscopically described, evaluated, and recorded. Processing was carried out at the Jesús Menéndez Avian Research and Diagnostic Laboratory (LIDA Jesús Menéndez), located in the Mulgoba neighborhood, Boyeros municipality, Havana province. Samples of liver, heart, and spleen were collected in sterile Petri dishes for bacteriological analysis, while the intestines were removed and submitted to the parasitology unit for helminthological examination.

Clinical and anatomopathological variables

The following clinical signs were recorded: diarrhea, ruffled feathers, comb cyanosis, comb retraction, emaciation, and somnolence. Macroscopic anatomopathological lesions related to digestive tract involvement were documented: catarrhal enteritis, typhlitis with gaseous distension, ovarian degeneration with deformed follicles, hepatic and splenic congestion, hemorrhage in the hepatic parenchyma, hepatic necrosis, hepatic degeneration, salpingoperitonitis, fibrinous polyserositis, hepatic atrophy, splenic atrophy, and ovarian atrophy. Some of these lesions can be seen in Figure 2. These findings were obtained from the clinicopathological records of cases examined in the pathology unit.

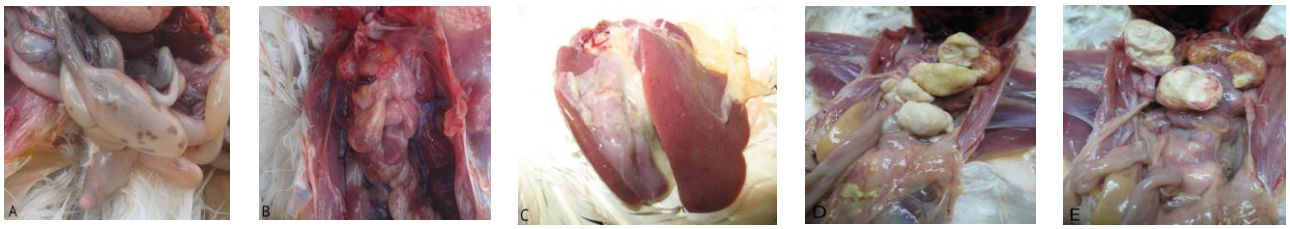

Figure 2. A. Typhlitis with gaseous distension B. Ovarian degeneration with deformed follicles C. Hemorrhagic liver. D–E. Fibrinous polyserositis and fibrinous perihepatitis.

Microbiological and parasitological variables

Results from the bacteriology and parasitology units were obtained for laying hens positive for Enterobacteriaceae, which included *Escherichia coli*, *Enterobacter* spp., *Pantoea agglomerans*, *Klebsiella* spp., *Citrobacter* spp., *Citrobacter freundii*, *Citrobacter amalonaticus*, *Yersinia intermedia*, *Yersinia enterocolitica*, and *Shigella boydii*, as well as coinfections involving 2, 3, and 4 bacterial species. Parasitic infections were identified as nematodes (*Subulura suctoria*) and cestodes (*Raillietina cesticillus*) from the case records of the evaluated farms.

Statistical analysis

Contingency tables were constructed with the clinical and general anatomopathological results from the 30 laying hens, organized by poultry farm in Excel spreadsheets. A chi-square analysis and the phi correlation coefficient (φ) were performed to evaluate the association between coinfections (1 — *Raillietina cesticillus* + *Enterobacteriaceae*; 2 — *Subulura suctoria* + *Enterobacteriaceae*; 3 — *Raillietina cesticillus* + *Subulura suctoria* + Enterobacteriaceae; 4 — Enterobacteriaceae) and each clinical sign (diarrhea, ruffled feathers, comb cyanosis, comb retraction, emaciation, somnolence). The lesions considered were: catarrhal enteritis, typhlitis with gaseous distension, ovarian degeneration with deformed follicles, hepatic and splenic congestion, hemorrhage in the hepatic parenchyma, hepatic necrosis, hepatic degeneration, fibrinous polyserositis, and ovarian atrophy. Statistical analyses were performed using IBM SPSS Statistics, version 27 (IBM Corp., 2020).

RESULTS AND DISCUSSION

The most frequent clinical signs were diarrhea (60%) and ruffled feathers (53.3%), while the least frequent were comb cyanosis (6.7%) and somnolence (3.3%). Among the lesions, catarrhal enteritis (70%), hepatic degeneration (56.7%), and typhlitis with gaseous distension (50%) were prominent, suggesting predominant digestive and hepatic involvement (Table 1).

Table 1. Frequency of clinical signs and anatomopathological lesions in White Leghorn hens affected by enterobacteria and helminths

Clinical signs	Positive hens	%
Diarrhea	18	60.0
Ruffled feathers	16	53.3
Comb retraction	7	23.3
Emaciation	4	13.3
Comb cyanosis	2	6.7
Somnolence	1	3.3
Anatomopathological lesions		
Catarrhal enteritis	21	70.0
Hepatic degeneration	17	56.7
Typhlitis with gaseous distension	15	50.0
Ovarian degeneration with deformed follicles	10	33.3
Hepatic necrosis	10	33.3
Splenic atrophy	7	23.3
Hemorrhage in the hepatic parenchyma	5	16.7
Hepatic atrophy	4	13.3
Fibrinous polyserositis	3	10.0
Salpingoperitonitis	2	6.7
Hepatic and splenic congestion	1	3.3
Ovarian atrophy	1	3.3

For the coinfection of *Subulura suctoria* + Enterobacteria with diarrhea, a significant association was observed ($\chi^2 = 8.17$; $p = 0.004$), and the coefficient indicates a moderate negative correlation ($\varphi = -0.52$); this suggests that this coinfection is associated with a lower incidence of diarrhea. However, for the coinfection of *Raillietina cesticillus* + Enterobacteria with ruffled feathers, there is a marginally significant association ($\chi^2 = 3.81$; $p = 0.051$) with a coefficient indicating a moderate positive correlation ($\varphi = 0.36$) (Table 2). Ruffled feathers could be an external indicator of physiological stress or an underlying pathology; this result suggests that the coinfection could contribute to that condition.

Table 2. Helminth and enterobacterial coinfections associated with clinical signs in White Leghorn laying hens

Coinfection of helminths and Enterobacteriaceae	Clinical manifestation	Chi-square (χ^2)	P value	Phi correlation coefficient (φ)
Raillietina cesticillus + Enterobacteria	Diarrhea	0.14	0.71	0.07
	Ruffled feathers	3.81	0.051	0.36
	Comb cyanosis	0.24	0.63	-0.09
	Comb retraction	0.67	0.41	-0.15
	Emaciation	0.37	0.54	-0.11
	Somnolence	0.12	0.73	-0.06
Subulura suctoria + Enterobacteria	Diarrhea	8.17	0.004	-0.52
	Ruffled feathers	0.20	0.65	-0.08
	Comb cyanosis	3.21	0.073	0.33
	Comb retraction	1.00	0.32	0.18
	Emaciation	0.99	0.32	0.18
	Somnolence	1.55	0.21	0.23

Ralletina cesticiillus + *Subulura suctoria* + Enterobacteria	Diarrhea	0.79	0.37	0.16
	Ruffled feathers	1.18	0.28	0.20
	Comb cyanosis	0.07	0.79	-0.05
	Comb retraction	0.21	0.65	-0.08
	Emaciation	0.12	0.73	-0.06
	Somnolence	0.04	0.85	-0.03

Only the coinfection *Subulura suctoria* + Enterobacteria shows significant associations ($p < 0.05$) with hepatic degeneration, ovarian degeneration, and ovarian atrophy, with the correlation coefficient indicating a similar moderate positive association among these lesions (Table 3).

Table 3. Coinfections of helminths and Enterobacteriaceae associated with pathological lesions in White Leghorn laying hens

Coinfection of helminths + Enterobacteriaceae	Anatomopathological lesion	Chi-square (χ^2)	P value	Phi correlation coefficient (φ)
Raillietina cesticillus + Enterobacteria	Catarrhal enteritis	3.499	0.061	0.342
	Typhlitis with gaseous distension	2.917	0.088	0.312
	Ovarian degeneration	1.667	0.197	0.236
	Hepatic and splenic congestion	0.115	0.734	0.062
	Hemorrhage in the hepatic parenchyma	0.667	0.414	0.149
	Hepatic necrosis	0.370	0.542	0.111
	Hepatic degeneration	0.739	0.390	0.157
	Fibrinous polyserositis	0.238	0.625	0.089
	Ovarian atrophy	1.667	0.197	0.236
Subulura suctoria + Enterobacteria	Catarrhal enteritis	0.009	0.925	0.017
	Typhlitis with gaseous distension	0.918	0.338	0.175
	Ovarian degeneration	6.429	0.011	0.463
	Hepatic and splenic congestion	2.414	0.120	0.284
	Hemorrhage in the hepatic parenchyma	0.286	0.593	0.098
	Hepatic necrosis	1.429	0.232	0.218
	Hepatic degeneration	6.212	0.013	0.455
	Fibrinous polyserositis	0.918	0.338	0.175
	Ovarian atrophy	6.429	0.011	0.463
Ralletina cesticiillus + *Subulura suctoria* + Enterobacteria	Catarrhal enteritis	3.399	0.065	0.337
	Typhlitis with gaseous distension	0.905	0.341	0.174
	Ovarian degeneration	2.069	0.150	0.263
	Hepatic and splenic congestion	0.036	0.850	0.035
	Hemorrhage in the hepatic parenchyma	0.207	0.649	0.083
	Hepatic necrosis	0.115	0.734	0.062
	Hepatic degeneration	0.791	0.374	0.162
	Fibrinous polyserositis	0.074	0.786	0.050
	Ovarian atrophy	2.069	0.150	0.263

Although no statistically significant associations were found, the lesions catarrhal enteritis and gaseous typhlitis show trends approaching significance and a moderate correlation, which suggests they may be related to Enterobacteriaceae infections. For the remaining lesions, correlations were weak or absent, indicating there is insufficient evidence to directly link them to these infections (Table 4).

Table 4. Association of Enterobacteriaceae infections with clinical signs and pathological lesions in White Leghorn layers

Clinical signs	Chi-square (χ^2)	P value	Phi correlation coefficient (φ)
Diarrhea	1.49	0.22	0.22
Anatomopathological lesion			
Catarrhal enteritis	3.320	0.068	0.333
Typhlitis with gaseous distension	3.519	0.061	0.343
Ovarian degeneration	1.364	0.243	0.213
Hepatic and splenic congestion	0.376	0.540	0.112
Hemorrhage in the hepatic parenchyma	0.545	0.460	0.135
Hepatic necrosis	0.076	0.783	0.050
Hepatic degeneration	0.151	0.697	0.071
Fibrinous polyserositis	0.779	0.377	0.161
Ovarian atrophy	1.364	0.243	0.213

The results of this research are novel when we consider that most studies in Cuba on Enterobacteriaceae coinfections in birds have been carried out in semi-rustic hens and in association with other pathologies. Rodríguez *et al*. (2018) did not find bacterial agents associated with helminths in semi-rustic hens, although a primary helminth infection can cause alterations of the intestinal mucosa and foster the conditions necessary for the establishment of a secondary infection by *E. coli*, since it is part of the normal intestinal flora and can spread to other organs of the bird.

Villalón *et al*. (2017) observed that the clinical-pathological picture of intestinal and cecal coccidiosis can be aggravated when there is coinfection with enterobacteria (*Salmonella* spp.). This suggests that coccidiosis would not only affect the intestine but could also trigger more severe systemic alterations. A similar finding was reported by Pacheco and Carrazana (2015), where the interaction between infectious and deficiency diseases, worsened by biosecurity problems, can lead to increased morbidity and mortality from enterobacteriosis. In that case, avian leukosis and the osteomalacia–osteoporosis syndrome associated with enterobacteriosis resulted in substantially greater impacts in commercial laying hens.

In a study conducted on semi-rustic hens from the provinces of Artemisa and Mayabeque, Rodríguez *et al*. (2016a) found that the starter category was affected by several species of Enterobacteriaceae. *Escherichia coli* was the most abundant species in the organs (liver, heart, and spleen) and across the production categories evaluated (starter, replacement, and breeders). Additionally, some of the birds presented mycosis caused by *Candida albicans*.

This study also detected parasitic coinfection by *Raillietina cesticillus* and *Subulura suctoria*. The presence of a single cestode species may be due to immunity conferred by one species against others, as suggested by Rashid *et al*. (2019). Secondly, the cage rearing system—where helminth prevalence is lower compared with floor-rearing systems—could explain this finding (Soulsby, 1987; Shifaw *et al*., 2021). *Raillietina cesticillus* is widely distributed in Cuba, affecting laying hens, breeders, semi-rustic and free-range birds (Hernández *et al*., 2002; Larramendy *et al*., 2006; Xuárez *et al*., 2011). In 2016 it was reported for the first time in wild quail (*Colinus virginianus*) in Cuba (Rodríguez *et al*., 2016b). The authors observed few anatomopathological lesions attributable to *R. cesticillus* and other helminths, which they ascribed to the low pathogenicity and low infestation rates. The same study also detected *S. suctoria* in the ceca of infested birds; although this parasite does not usually produce obvious clinical signs (Sánchez, 2010), it is important to consider that the degree and extent of enteric lesions correlate with parasite burden (Rashid *et al*., 2019).

The clinical signs and macroscopic lesions identified, though general and nonspecific, are consistent with other reports of parasitism by *R. cesticillus* and *C. infundibulum* in semi-rustic hens from Cuba. Lesions caused by both cestodes also include intestinal congestion, bone fragility, and loss of cartilage from the femoral head (Rodríguez *et al*., 2018). In other tropical regions, *R. cesticillus* has been found in mixed infection with *Capillaria* spp. in the small intestine (Salawu and Emmanuel, 2023). A study in Japan reported high mortality in laying hens due to severe *R. cesticillus* parasitism. The decline in egg production was attributed to impaired nutrient absorption caused by *R. cesticillus* (Oshima *et al*., 2024).

The results obtained are consistent with Nolan *et al*. (2013) and Hassan (2024). Colibacillosis caused by avian pathogenic *Escherichia coli* (APEC) is characterized by numerous lesions that range from acute to chronic forms depending on disease presentation, and may include colisepticemia, hemorrhagic septicemia, coligranuloma (Hjarre disease), airsacculitis, swollen head syndrome, venereal colibacillosis, coliform cellulitis, enteritis, peritonitis, pericarditis, salpingitis, orchitis, osteomyelitis/synovitis, panophthalmitis, omphalitis, and enteritis. Colibacillosis should be differentiated from fowl cholera, pullorum disease, and chronic respiratory disease.

At the experimental level in Cuba, Colas *et al.* (2014) determined that inoculation of *Ornithobacterium rhinotracheale* and *E. coli* in five-week-old laying hens produced macroscopically more evident lesions and greater hepatic degeneration compared with groups inoculated with a single bacterial agent. This finding suggests that, under field conditions where birds may be exposed to multiple pathogens simultaneously, the impact on their health can be much more severe.

Although early exposure to certain Enterobacteriaceae such as *Citrobacter*, *E. coli*, and *Salmonella* can cause mild enteritis in two-week-old chicks without affecting growth, it may or

may not modify inflammatory parameters, thereby influencing the birds' ability to respond to other pathogens (Chasser *et al*., 2003, 2021).

Akporeha *et al*. (2025), in a case report of colibacillosis in Nigeria, found high mortality in laying hens accompanied by postmortem lesions compatible with Newcastle disease, suggesting a possible coinfection. Findings included biliary sludge, airsacculitis, ascites, hepatic congestion, a friable liver, and petechial hemorrhages in the proventriculus. Although Newcastle disease was not included in the diagnosis because the birds had been previously vaccinated, the researchers did not rule out the possibility that Newcastle disease acted as a predisposing factor for colibacillosis.

Some enterobacterial coinfections may be underestimated and can mask the diagnosis. In Indonesia, broiler chickens exhibited very general signs such as lethargy, anorexia, abdominal distension, and whitish diarrhea that led to death. Necropsy combined with histopathological and bacteriological analysis of specific organs confirmed coinfection with *Shigella* sp. and *E. coli*. (Ardiana *et al.*, 2024).

Shi *et al*. (2014) found that the route of administration of *Shigella* spp. in chicks can influence the clinical and pathological manifestations. Via intraperitoneal inoculation they observed depression, weakness, somnolence, drooping wings, comb retraction, ruffled feathers, anorexia, immobility, diarrhea, and death. Lesions were accompanied by hepatic degeneration, jaundice, hemorrhages in the heart, lungs, and spleen; intestinal edema and gaseous typhlitis. In contrast, no significant clinical signs or deaths were observed in groups inoculated via the crop.

CONCLUSION

The findings of this study underscore the importance of managing bacterial and parasitic coinfections in laying hens to reduce the incidence of severe lesions that compromise productivity and animal welfare, with a One Health approach. The preliminary results open opportunities to increase the sample size per poultry farm and to incorporate molecular techniques for more precise characterization of the pathogens involved.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: MCC, OCM, KBC, MAP, DRG; data analysis and interpretation: MCC, OCM, KBC, MAP, DRG, GGA; writing of the manuscript: MCC, MAP, DRG, GGA.

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.