An Acad Bras Cienc (2024) 96(2): e20230815 DOI 10.1590/0001-3765202420230815
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ANIMAL SCIENCE

Anatomopathological changes in laying quails

DÉBORA C. NEIVA, MATHEUS J. DOS S. CANDIDO, AMANDA A. ASSIS, THAIS G. TAVARES, MÁRCIO P.A. CORREIA, EMERSON A. DE O. SOUZA, DIRLEI M. DONATELE, IGOR C.K. DA CRUZ, ISABELLA V.F. MARTINS, MARIA A. DA SILVA & JANKERLE N. BOELONI

Abstract: The objective of the current study was to carry out a survey of the main anatomopathological alterations in raising quails and evaluate possible interference of these in the bone tissue. To obtain the data, 23 quails were collected from farm in the central Serrana region of Espírito Santo. Necropsies with macroscopic descriptions, microbiological, coproparasitological, radiographic and histomorphometric tests were carried out. It was done data descriptive analysis and average comparision using Student T test. It was found that they presented lesions predominantly in the digestive system, followed by urinary and reproductive, and muscular system, were the altered color of the liver (47%) was the most frequent lesion. In the parasitological exams, it was found oocysts of *Eimeira* sp. (39.13%). In the microbiological exams, it was detected predominantly *Escherichia coli* (83%). Moderate osteopenia in quails, but the percentage of trabecular bone on bones was similar between healthy and diseased quails, without bone changes in histology. Microscopically, it was observed lung congestion as predominant lesion. It is concluded that there was predominance of alterations in the digestive system and mild parasitic infection; and although there was moderate level of osteopenia, there wasn't bone change as a result of the observed infections.

Key words: Bacterial diseases, bone tissue, laying hens, parasitism, poultry farm.

INTRODUCTION

Domestic birds are among the animals targeted by anatomopathological studies, considering their great economic relevance (Nascimento et al. 2021). Quails are among the most widely used layers in Brazilian poultry farming, producing 315.6 million dozen eggs (IBGE 2019). In Brazil, the state of Espírito Santo is currently considered the biggest producer of quail eggs (ABPA 2021, IBGE 2019). Therefore, it is important to carry out surveys of the main diseases of laying birds in Espirito Santo and their etiologies to improve the development of poultry farming in the state, since there are not yet so many studies in this region (Souza 2012).

Due to the intensive and semi-intensive production systems of layers for greater egg production, these birds are more susceptible to various bacterial, fungal, viral and metabolic diseases that affect one or more systems, such as respiratory, reproductive, bone and digestive (Melo et al. 2018). Although the occurrence of these diseases is caused by the increase in the amount of pathogens in the body, it can also be facilitated by inadequate nutrition, management and hygiene of birds, and generate consequences from discomfort and suffering of the animal to economic losses for the poultry farmer (Souza et al. 2017). Taking into account that there are few studies of this area in the state of Espírito Santo, the objective of the present work was to

survey the main anatomopathological changes in domestic quail in intensive rearing systems in farms of Espírito Santo, in addition to relating the diseases, mainly bacterial, with possible changes in bone tissue. It is important to point out that this study brings unprecedented and relevant results.

MATERIALS AND METHODS

The current study was developed in the Animal Pathology Sector, in the Animal Parasitology Laboratory, Animal Microbiology Laboratory and Imaging Diagnosis Sector, all located in the Veterinary Hospital of the Center for Agricultural Sciences and Engineering of the Federal University of Espírito Santo (CCAE/UFES), with all experimental procedures approved by CEUA Alegre with protocol No. 014/2020.

Obtaining the birds

The collection of the birds took place in November 2021. Initially, 23 Japanese quail (*Coturnix coturnix japonica*) previously vaccinated with autogenous vaccines and with clinical signs originating from an intensive quail breeding farm located in Santa Maria de Jetibá, Espírito Santo, were collected. Among these, 10 were 42 weeks old, while the remaining 13 were on average 21 weeks old and were in a non-air-conditioned environment. All quails presented at least one of the following clinical signs: apathy, white diarrhea, loss of appetite, abnormal grouping, bristling feathers, cloaca with intense accumulation of feces, large amount of malformed eggs and reduction in feed intake.

Necropsy and histological evaluation of the organs

The birds were transported to the Veterinary Hospital of UFES Alegre, where they were euthanized by cervical displacement. Subsequently, necropsy of all animals was performed according to the technique adopted in the Animal Pathology Sector of UFES. During the autopsy, external and internal examinations were performed with descriptions of the alterations found. In addition, tissue fragments were collected from various organs (trachea, lung, heart, spleen, gizzard, liver, small [duodenum] and large intestines, pancreas, ovary, oviduct, bursa of Fabricius and brain) and also from long bones (right femur and tibiotarsus).

All tissue fragments of the organs and bones were placed in 10% buffered formalin for fixation and subsequent routine processing of paraffin inclusion for histopathological analysis, and the long bones were decalcified in EDTA (described later) before routine processing. After processing, the materials were cleaved in sections of 4µm thickness and the slides were staining by Hematoxylin and Eosin (HE) and, when necessary, special periodic Schiff Acid Staining (PAS) was also performed, depending on the diagnostic suspicion. After staining, the slides were observed under an optical light microscope for descriptive analysis of the findings, with subsequent compilation of data and photomicroscopy using an image capture microscope (Opticam LOPT14003 0500R).

Parasitological and microbiological examinations

During the autopsy, feces were collected directly from the large intestine and placed in plastic bags, identified and then refrigerated, for posterior coproparasitologic evaluation through optical microscopic (100x magnification). The chosen technique was the Simple Fluctuation Centrifugal – CFS (Sheather 1923). The positive samples for oocysts were submitted to the addition of 2.5% aqueous potassium dichromate (K2Cr2O7) and kept in 20 mL test tubes for sporulation. Every two days, by adding a drop

of the sample between lamina and coverslip, the number of sporocysts and sporozoites was analyzed to determine the gender of the involved protozoan.

Also during the necropsy, it was used swabs to collect purulent exudates, when present, in several regions such as the ocular region, oviduct, intestine and air sacs of the birds. The swabs were refrigerated for later microbial testing. Then, inoculation was performed in blood agar medium and McConkey, gram staining, oxidase and catalase tests, inoculation in Indole Sulfide Motility agar (SIM), Klinger Iron agar, Triple Iron Sugar agar (TSI) and Phenylalanine agar.

Radiographic and morphometric evaluations of the long bones

From the collection of the long bones (right femurs and tibiotarsi) during necropsy, they were fixed in 10% buffered formalin, followed by dissection. After this procedure, the bones were submitted to radiographic examinations for subsequent evaluation of the degree of osteopenia (mild, moderate, and intense) according to the level of radiopacity and the integrity of the periosteum. The radiographic examinations were performed in an x-ray machine (emitter) of the brand CDK, model DIAFIX Microprocessed 500mA/125kV, with digitizer system of images of the type computed radiography (CR), brand Fujifilm, model FCR XLII Capsule.

After radiographic examinations, femurs and tibiotarsi were weighed using a precision scale (Toledo brand). Length, perimeter of proximal and distal epiphyses, as well as diaphysis were also evaluated with the aid of a millimeter measuring tape. After obtaining the weight and length of the bones, the Seedor Index (SI) was calculated, which corresponds to the weight of the bone (mg), divided by its length (mm) to evaluate bone density (Seedor et al. 1991).

Before the routine processing of paraffin inclusion, the long bones were submitted to the decalcification process, since the mineral present in the bones prevents the processing steps. Thus, for the decalcification, it was used a descaling solution composed of monobasic sodium phosphate (31.2 g in 1L of water - solution 1), bibasic sodium phosphate (28.4 g in 1L of water - solution 2), and EDTA disodium salt (140 g). The final solution consisted of the following mixture: 140 g of EDTA + 280 mL of solution 1 + 720 mL of solution 2 + 1L of water. The solution was replaced twice a week until complete decalcification, which lasted 45 days, and the degree of demineralization was followed by periodic radiographs.

With the demineralized bones, cleavage was performed and then the routine processing of paraffin inclusion, cut into 4 µm, submitted to HE staining and, subsequently, observed under an optical microscope to perform the descriptive analysis and morphometric analysis of the percentage of trabecular bone. The morphometric analysis was performed in the epiphysis of all femurs and tibiotarsus, in the objective of 20x, in seven fields each, with the aid of a graticule with 99 points taken throughout the length of the histological section.

Statistical analysis

For the data statistical analysis, the Student's T test was performed with 5% significance, to compare the percentage of trabecular bone of healthy birds (without bacterial infection) with those diseased (with bacterial infection). The other analyses performed were performed descriptively.

RESULTS

Microscopic findings

Regarding the macroscopic findings, 82.5% of the quails had at least some type of lesion on external examination, being that the most frequent being was the extension of white and softened feces of the large intestine accumulating in the cloaca indicating diarrhea (30%), followed by rarefaction of feathers on the dorse (common alteration in layers) (21%), eye lesion such as unilateral corneal opacity (13%), calluses in phalanges (13%), increased abdominal volume (9%), supracranial cutaneous nodule (4%) and cloacal prolapse (4%). It is important to emphasize that the total number of lesions is not equivalent to the number of birds, because some quails presented more than one alteration.

In the internal examination, it was evidenced that all quails presented at least one macroscopic alteration in some organ/system/site, and all quails (100%) presented at least one lesion in the digestive system, 91% in the urinary and reproductive systems, 78% in the muscular system, 56% in the hematopoietic/lymphatic/lymphoid system, 30% in the cardiorespiratory system, and 8% in the bone system. The main lesions are presented in Figure 1 and the others are presented in detail in Table I. In this sense, the most observed alterations were liver disease characterizing the altered color (yellowish, white or blackish) of the liver (47%) (Figure 2d and e) and hypotrophy of the pectoral muscles, leaving the keel of the sternum in evidence (47%). Followed by the diffuse pallor of the spleen (39%), fibrin deposition with an adjacent cyst in the oviduct (39%), congestion of the serous of small intestine (35%), ovarian hypotrophy (35%), ascites (9%), and femur fracture associated with hemorrhage (8%).

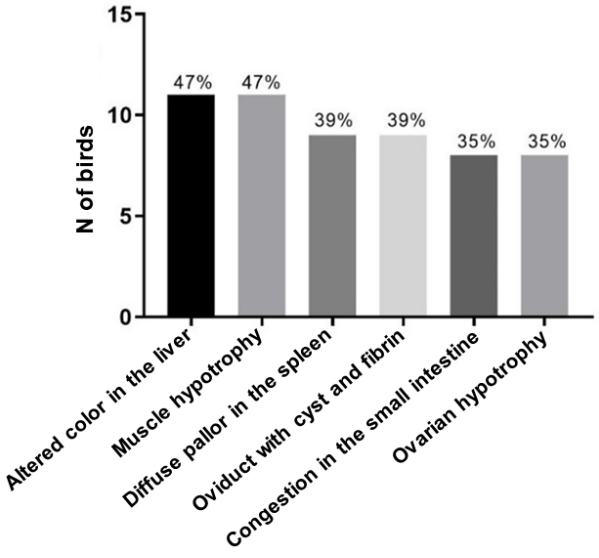

Figure 1. Main lesions found in the internal examination during the necropsy of Japanese quail.

Parasitological findings

Parasitological examinations of quail revealed oocysts of *Eimeria* sp. coccidia in 39.13% of quail, which presented mild infection (1 to 10 oocysts).

Microbiological findings

The microbiological analyses were performed on 12 samples (caseous or fibrinous exudates) from different sites, so the predominance of the bacterium *Escherichia coli* (83%) was detected, affecting several sites/organs such as small intestine, coelomic cavity, ovary, pericardial sac, lung and air sacs with the formation of caseous and fibrinous contents and malformed eggs. In addition, it was also recorded the growth of the bacterium *Klebsiella* spp. collected from a yellowish thickening in the wall of the duodenum of a bird (8.3%), and *Pseudomonas aeruginosa* together with *Salmonella* spp. in the coelomic cavity with fibrin and in a supracranial cutaneous nodule with a total frequency of 16.6%. The microbiological findings are summarized in Table II.

Table I. Main affected organs and macroscopic changes observed in Japanese quail.

Organ/alteration	Absolute value	Relative value
Liver	18/23	78%
Altered coloration	11/23	47%
yellowish areas	5/23	21%
white areas	5/23	21%
Blackened organ	1/23	4%
Diffuse congestion	7/23	30%
Hepatomegaly	3/23	13%
Skeletal striated muscle	18/23	78%
Muscle hypotrophy (keel overt)	12/23	52%
Carcass pallor	6/23	26%
Bleeding areas	5/23	21/%
Spleen	16/23	70%
Diffuse pallor associated with white pulp hyperplasia	9/23	39%
Splenomegaly	4/23	17%
Blackened areas	1/23	4%
Bleeding áreas	1/23	4%
Diffuse congestion	1/23	4%
Small intestine	11/23	52%
Serous congestion	8/23	35%
Wall thickening due to edema	6/23	26%
Fecaloma causing intestinal rupture	1/23	4%
Elevated plaques with mucoid contents	3/23	13%
Oviduct	10/23	43%
Serous cyst + diffuse fibrin deposition	9/23	39%
Mucosal congestion	2/23	9%
Kidney	9/23	39%
Congestion	6/23	26%
White areas	2/23	9%
Pallor	1/23	4%
Ovary	8/23	35%
Ovarian hypotrophy	8/23	35%
Malformed eggs in the uterus	3/23	13%
Heart	3/23	13%
White areas	2/23	9%
Fibrin in the pericardium	1/23	4%
Lung	2/23	9%
Congestion	1/23	4%
Fibrin adhesion	1/23	4%
Trachea	2/23	9%
Yellowish content	1/23	4%
Caseous exudate	1/23	4%
Others	3/23	13%
Tongue with yellowish focal area	1/23	4%
Brain with congestion	1/23	4%
Esophagus with intense pallor	1/23	4%

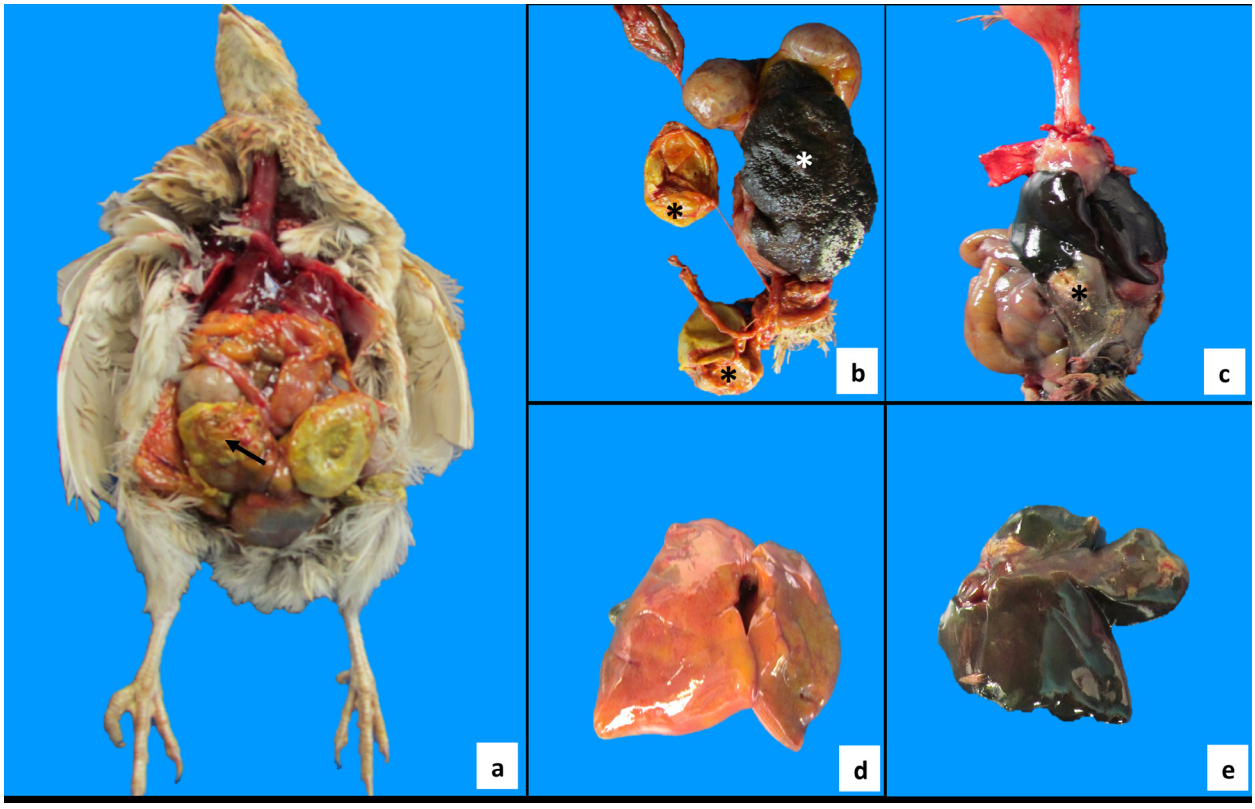

Figure 2. Lesions found during the autopsy of Japanese quail. a) Deposition of diffuse fibrin in the serosa of the oviduct (arrow). b) Fecaloma (white asterisk) focally extensive, severe and malformed egg (black asterisk). c) Discrete focal caseous exudate (*) at the caudal edge of the liver. d) Liver with multifocal to coalescent areas yellowish in moderate amount. e) Liver diffusely blackened in moderate amount.

Table II. List of sites and lesions where swabs were collected for microbiological tests, infectious agents found and their frequencies in Japanese quail.

Collect/alteration site	Infectious agent	Frequence
Serosum of the duodenum	*Klebsiella* spp.	8.3%
Interior of ovarian follicle	*Escherichia coli*	75%
Serosum of the duodenum		
Coelomatic cavity with fibrin		
Elevated plaque of the intestine wall		
Air sac		
Pericardial sac		
Shapeless and flacid ovary		
Coelomatic cavity with fibrin	*Escherichia coli*, *Salmonella* spp.; *Pseudomonas* spp.	8.3%
Supracranial cutaneous nodule	*Salmonella* spp.; *Pseudomonas* spp.	8.3%

Bone morphometry and radiographic analysis

Regarding quail bone parameters (Table III), the mean values of Seedor Index (SI) were 22.85 mg/mm in the femur and 23.56 mg/mm in the tibiotarsus. Among the degrees of osteopenia observed in the radiographic analysis (Figure 3), the most frequent was moderate degree. The mean percentage of trabecular bone (Figure 4) in the femoral epiphyses was 22.1% in quails without bacterial diseases (healthy), and 25.96% in quails with bacterial diseases (diseased), with no statistical difference between the means (p=0.4142). While in the tibiotarsus of healthy quails, the mean percentage of trabecular bone was 27.64% and in diseased quails it was 24.77%, also not differing statistically (p=0.4275). Regarding the histological evaluation of the bone tissue of femurs and tibiotarsi of quails, there was a predominance of trabeculae discretely connected to each other, osteocytes and osteoblasts were sometimes cuboid, sometimes flattened, and osteoclasts were rare

Table III. Seedor index, degree of osteopenia and percentage of trabecular bone of the epiphyses of femurs and tibiotarsi of Japanese quail. *Diseased birds. Discrete: +; Moderate: ++; Intense: +++.

Quail	Femur			Tibiotarsus		
	Seedor Index	Degree of osteopenia	% Trabecular bone	Seedor Index	Degree of osteopenia	% Trabecular bone
1*	23.68	+	15.66	22	+	20.88
2*	22.5	++	27.02	16	++	25.25
3*	20	++	26.77	18.75	++	14.65
4*	24.32	++	-	25	++	32.58
5*	18.75	+++	39.15	41.18	+++	-
6	22.45	++	32.58	26	++	10.35
7	18.42	++	15.66	19.57	++	12.37
8*	23.08	++	25.5	24	++	42.42
9	28.95	+	-	31.91	+	27.27
10	29.73	++	28.79	25.53	++	37.37
11	26.32	++	26.26	29.17	+	24.75
12*	20	++	28.79	20	+	30.55
13*	27.5	++	26.77	21.57	++	26.01
14	23.08	+	23.99	23.40	+	18.43
15	28.21	+++	-	29.17	+++	25.75
16	15.15	++	-	19.15	++	25.50
17*	23.08	+++	42.93	17.02	+++	22.47
18*	22.22	+++	31.31	20.83	+++	31.06
19*	28.21	+++	29.04	20.83	+++	30.55
20	15.15	++	-	26.53	++	35.35
21	24.32	+	13.38	20.83	+	23.74
22	17.65	+	-	20	+	31.56

in all tibiotarsi and in the most of femurs, being found in moderate quantities in Howship's lacunae in only one femur. No bone changes were found in quails (Figure 5).

Microscopic findings

Regarding the histopathological analysis, it was found that, as in the macroscopic evaluation, all quails had at least one histological alteration. All quails presented lesions in the digestive system (100%), which is the most affected system. In addition, 78% had lesions in the hematopoietic/lymphatic/lymphoid system and 74% of the quails had lesions in the urinary and reproductive systems. In relation to the frequency of histopathological lesions observed (Figure 6), pulmonary congestion (91%) and hemorrhage (78%) were the most frequent, followed by multifocal renal congestion (70%), random hepatic congestion (61%), hyperplasia of the white pulp of the spleen (43%) and inflammatory infiltrate (lymphocytes and histiocytes) in the serosa of the small intestine (26%). The details of the microscopic lesions in the various organs and sites of the organism are represented in Table IV. Some of the microscopic findings in the small intestine, trachea and lung, spleen and bursa of Fabricius of Japanese quail are presented in Figures 7, 8 and 9, respectively.

DISCUSSION

In order to get to the possible diagnoses of the present study, it was necessary to associate the macroscopic findings with the histopathological ones, along with the bacteriological and parasitological exams, as performed by Lopes (2021). The lesion most frequently observed on

Figure 3. Radiographic evaluation of the long bones (femurs and tibiotarsi) of Japanese quail. The degree of moderate osteopenia was the most predominant. The numbers 3, 4, 6, 7, 8, 9, 10, 11, 12 and 13 represent the quails evaluated.

external examination was white and softened stools from the large intestine accumulating in the cloaca which is an indication of diarrhea. It is important to emphasize that this is a recurrent symptom of several enteric diseases caused by infectious agents such as Rotavirus and Coronavirus, and bacteria such as *Salmonella enterica* and *Escherichia coli* (Naranjo 2011). In addition, the lesions most commonly found on internal examination were liver disease causing changes in liver color followed by muscle hypotrophy.

In this way, associating the high-frequency microbiological results of the *E. coli* bacterium with the compatibility in the histopathological findings, the predominant disease in quail was suggestive of colibacillosis. According to Almeida et al. (2020), birds affected by colibacillosis present several lesions in several systems, mainly in the digestive system, as well as observed in the study in question. Colibacillosis can be systemic or localized, affecting poultry such as chickens, quail, turkeys and ratites of all ages. Its localized form can cause perihepatitis, aerosaculitis, omphalitis, salpingitis, coligranuloma, oophoritis, pericarditis, osteomyelitis, complicated chronic respiratory disease, among others (Ferreira & Knobl 2020, Lopes 2021, Nolan et al. 2020).

Most of the findings of this study are compatible with the symptoms of colibacillosis, being found localized forms of this disease, such as salpingitis, coligranuloma and oophoritis, and its systemic form (coliseptisemia) as cited by Ferreira and Knobl (2020). All quails in the present study that were infected by *E. coli* presented at least one of the following liver lesions: cytoplasmic vacuolization (hepatocellular degeneration) resulting in multifocal white and yellowish areas, inflammatory infiltrate (lymphocytes and histiocytes), areas of necrosis associated with bacterial lumps, diffuse congestion and hepatomegaly, being considered common alterations resulting from colibacillosis (Casagrande et al. 2017, Leão et al. 2018, Lopes 2021, Martins et al. 2017). The liver was the organ most affected by colibacillosis, similar to the result observed by Casagrande et al. (2017).

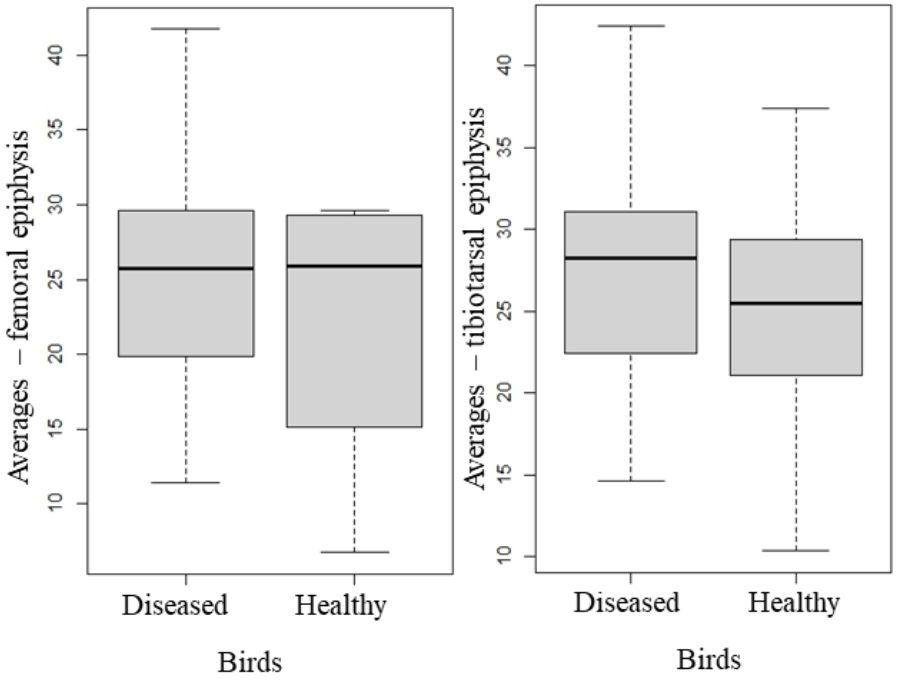

Figure 4. Average and standard deviation of the percentage of trabecular bone of the epiphyses of the femur and tibiotarsus of diseased and healthy quail. The comparison of averages was performed using the Student's t-test at 5% significance.

Hypotrophy of the pectoral muscles was also very evident in quails (resulting from weight loss), along with opacity of the air sacs, hyperemia of the intestinal mucosa, splenomegaly and pallor of the spleen, among others.

According to Barnes et al. (2008), such symptoms are the result of colisepticemia in birds. The main histological findings in quail, such as hemorrhage and pulmonary congestion can be justified by the involvement of *E. coli* in the respiratory tract, causing "complicated chronic respiratory disease", which may lead to pneumonia, pleuropneumonia as well as tracheitis and airsacculitis in fowls (Ferreira & Knobl 2020). Specifically in the spleen, quails with *E. coli* presented splenomegaly with white pulp hyperplasia and eosinophilic inflammation, as well as hemorrhagic areas, congestion and hemosiderosis.

Fibrin deposition with adjacent cyst in the oviduct and mucosal congestion can be explained by the predisposition of adult birds to inflammation of the oviduct (salpingitis) by *E. coli*. The production is largely responsible for this predisposition, since this enterobacterium manages to colonize the oviduct when the level of estrogen is high, in the laying phase of the birds (Ferreira & Knobl 2020). The ovary is also one of the main sites of development of *E. coli*., causing oophoritis and characterizing ovarian infection (Ferreira & Knobl 2020, Melo et al. 2018), which lead to malformed eggs, ovarian hypotrophy and falling oviposition. Oophoritis can be occurred from fecal contamination by intestinal rupture (Melo et al. 2018), as observed in one of the quails with *E. coli*.

In addition to intestinal rupture, there were other macroscopic and histological changes in the small intestine, such as mucosal thickening with elevated plaques, hyperemia and congestion that were also associated with colibacillosis. Importantly, although strains of *E. coli* naturally exist in the intestines of birds, pathogenic strains cause rupture of the intestinal epithelium inducing pseudomembranous or ulcerative enteritis (Barnes et al. 2008). In the present work it was not performed the identification of pathogenic strains, via PCR, to identify their genetic material, but the microbiologial

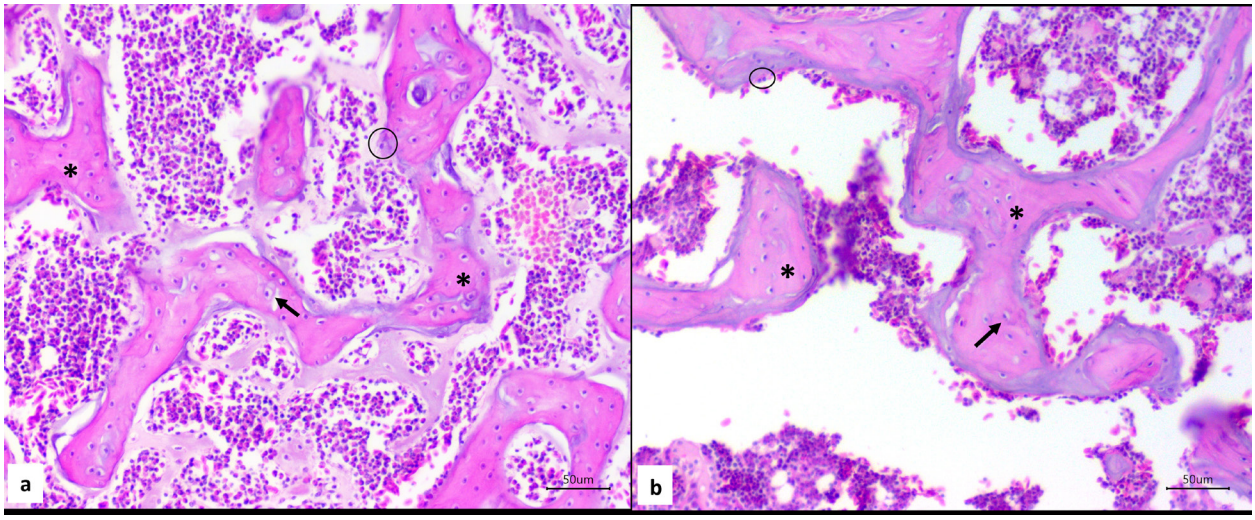

Figure 5. Photomicroscopies of femur (a) and tibiotarsal (b) of Japanese quails showing a predominance of trabeculae discreetly connected to each other (*), osteocytes (arrows) and osteoblasts (circles) sometimes cuboids, sometimes flattened, and osteoclasts were rare in all tibiotarsals and in most femurs. Hematoxylin and eosin, 40X increase.

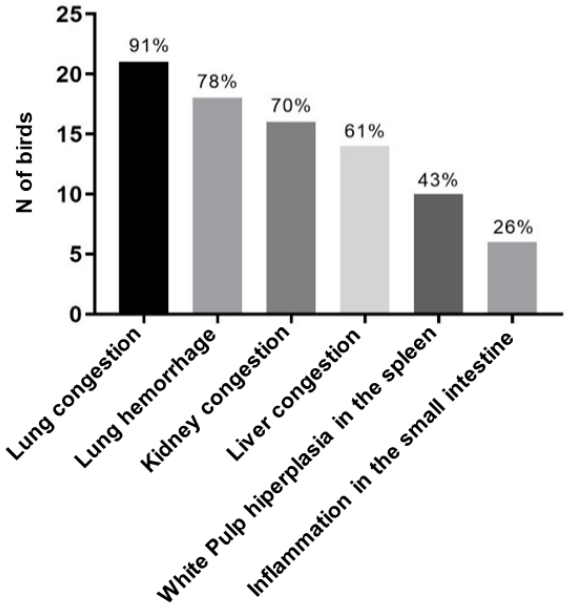

Figure 6. Main histopathological changes (percentage) in the organs collected from Japanese quail.

identification was sufficient to associate with the lesions found.

Another disease observed in quails was Salmonellosis. As well as quails affected by colibacillosis, those infected by *Salmonella* spp., along with *E. coli* and *Pseudomonas* spp. also presented liver lesions such as diffuse congestion, hepatomegaly and hepatocyte degeneration. These lesions result from the activation of phagocytes defense against *E. coli* and *Salmonella* spp. releasing specific substance to cause vacuolar degeneration of hepatocytes (Ito & Miyaji 2020, Martins et al. 2017). Lymphoid hyperplasia and hypertrophy can be justified both by Salmonellosis and by the application of autogenous vaccines in quails, and according to Freitas Neto et al. (2020) and Leão et al. (2018), this organ can also present splenomegaly, white areas (BPH) and hyperemia due to Salmonellosis. Both quails infected with Salmonellosis had cysts with material in the coelomic cavity, which is also a commonly found alteration in this disease (Freitas Neto et al. 2020). The clinical signs found in quails such as bristling feathers, white diarrhea, apathy, high initial mortality, decrease in feed consumption and posture are symptoms of both colibacillosis and Salmonellosis, being differential diagnoses among themselves and therefore requiring microbiological tests for confirmation (Freitas Neto et al. 2020, Santos et al. 2008). From the bacterial diseases observed, it is important to emphasize that *E. coli*, being secondary and opportunistic, is often associated with other infectious agents, such as the concomitant infection with *Salmonella* spp. It can also be found along with *Mycoplasma*, *Pasteurella*, *Avibacterium*, among others, and can be aggravated by co-infections (Ferreira & Knobl 2020, Taunde 2021).

In addition, colibacillosis is one of the most important diseases in poultry farming, considering that, like salmonellosis, it is a zoonosis of great relevance to public health, and can be triggered by any environmental, nutritional or infectious factor and can cause high mortality in the grange (Lopes 2021, Ferreira & Knobl 2020, Taunde 2021), as was the case with quails in this study. The means of prevention of colibacillosis and salmonellosis are related to the use of adequate vaccines and proper management such as: efficient ventilation to avoid the accumulation of ammonia and dust in the grange, hygiene for the supply of water and feed, temperature control, increase in the frequency of egg collection (Barnes et al. 2008, Casagrande et al. 2017, Ferreira & Knobl 2020), all these measures must be adopted appropriately by the poultry farm of the evaluated quail, mainly because it is an intensive breeding system. In addition, it is important to point out about the care with the spread of salmonellosis avoiding contact with transmitters such as birds, rodents and insects (Freitas Neto et al. 2020).

The occurrence of *Eimeria* spp. was also recorded in birds, however, besides the degree

Table IV. Histopathological changes of organs collected from Japanese quail.

Organ/alteration	Absolute frequence	Relative frequence
Lung	21/23	91%
Congestion	21/23	91%
Diffuse intense	11/23	48%
Multifocal intense	4/23	17%
Interstitial hemorrhage	18/23	78%
multifocal intense	10/23	43%
multifocal moderate	5/23	22%
Multifocal necrosis	2/23	8%
Spleen	18/23	78%
White pulp hyperplasia	10/23	43%
Multifocal discrete	4/23	17%
Multifocal moderate	6/23	26%
Moderate multifocal congestion	5/23	22%
Multifocal hemosiderosis	3/23	13%
Lymphoid sheath hypertrophy	1/23	4%
Necrosis of serosa fat	1/23	4%
Amorphous and eosinophilic material	1/23	4%
Liver	17/23	74%
Random congestion	14/23	61%
multifocal discrete	7/23	30%
multifocal moderate	5/23	22%
Random degeneration of hepatocytes	5/23	22%
Multifocal discrete	3/23	13%
Lymphohistiocytic/heterophilic inflammatory infiltrate	3/23	13%
Necrosis in serous	2/23	8%
Amorphous material between the hepatocyte cords	1/23	4%
Multifocal hemorrhage	1/23	4%
Kidney	17/23	74%
Congestion	16/23	70%
multifocal moderate	11/23	48%
multifocal discrete	5/23	22%
Multifocal hemorrhage	5/23	22%
moderate	3/23	13%
discrete	2/23	8%
Bacterial lumps in the pelvis	1/23	4%
Small intestine	14/23	61%
Lymphohistiocytic/heterophilic inflammatory infiltrate	6/23	26%
Serous necrosis associated with bacterial lumps	5/23	22%

Table IV. Continuation.

Discrete multifocal congestion	4/23	17%
Lymphoid tissue hyperplasia	1/23	4%
Structures compatible with *Eimeria* spp. meronts	1/23	4%
Large intestine	8/23	35%
Lymphohistiocytic/heterophilic inflammatory infiltrate in serous	4/23	17%
Moderate focally extensive mucosal necrosis	1/23	4%
Serous with intense multifocal necrosis	1/23	4%
Table IV. Histopathological changes of organs collected from Japanese quail (continuation).		
Heart	8/23	35%
Discrete multifocal lymphohistiocytic inflammatory infiltrate in the pericardium	4/23	17%
Discrete multifocal congestion	4/23	17%
Pancreas	7/23	30%
Discrete multifocal congestion	3/23	13%
Lymphohistiocytic inflammatory infiltrate	2/23	9%
Traqueia	6/23	26%
Discrete multifocal hyperplasia of lymphoid tissue	3/23	13%
Mucosal squamous metaplasia	1/23	4%
Submucosal hemorrhage	1/23	4%
Cloaca	5/23	22%
Moderate multifocal lymphohistiocytic inflammatory infiltrate	4/23	17%
Lymphoid rarefaction associated with edema	1/23	4%
Ovary	3/23	13%
Severe multifocal hypotrophy	1/23	4%
Moderate multifocal lymphohistiocytic inflammatory infiltrate	1/23	4%
Granuloma	1/23	4%
Oviduct	2/23	8%
Moderate multifocal congestion	2/23	8%
Moderate multifocal lymphohistiocytic inflammatory infiltrate	1/23	4%
Others	3/23	13%
Pectoral skeletal striated muscle with mild multifocal hypotrophy	1/23	4%
Mucosa of the apical region of the tongue with moderate focal area of necrosis associated with bacterial lumps (glossitis)	1/23	4%
Supracranial cutaneous nodule with severe focally extensive eosinophilic and necrotic material associated with bacterial lumps (dermatitis)	1/23	4%

of infection being mild, those that presented alterations in the small intestine were also affected by other agents, such as *E. coli*, *Salmonella* spp., *Pseudomonas* spp. or *Klebsiella* spp. and this may have masked the effects of coccidiosis. Those with exclusive infection by *Eimeira* spp. did not present intestinal alterations, except one, which presented blackened feces in the intestine suggestive of melena, along with thickening of the mucosal wall and fibrin in intense quantity and pancreas with inflammatory infiltrate (lymphocytes and histiocytes). Martins et al. (2017) reports granulomatous enteritis and diffuse acute necro-hemorrhagic typhlitis as one of the macroscopic changes that can be caused by coccidiosis.

According to bone parameters, it was possible to notice that the mean values of the Seedor Index were relatively higher, compared to those found in other studies, with values ranging from 10.5 to 19 mg/mm in the femur, and from 9.5 to 18.5 mg/mm in the tibiotarsus (Benites et al. 2020, Freitas et al. 2013, Gouveia et al. 2020). In previous studies, it was confirmed that as the level of sodium and vitamin A is increased in the diet, the Seedor Index (representing bone density) and bone strength increases consequently, proving the direct interference of adequate feeding of these nutrients on the bone quality of quails (Benites et al. 2020, Freitas et al. 2013). Benites et al. (2020) point out that quails with low SI have the least dense bone, which can generate a tibiotarsal dyschondroplasia if their weight increases.

It can be noted that there was no significant difference between the amount of trabeculae in the bones of diseased and healthy birds. As well as the degree of osteopenia and the histological descriptions of the tissues, being very similar between the bones. It is important to realize that the poorly connected and more distant bone trabeculae are characteristic of the pneumatic bone of birds (Konell et al. 2018). In addition, there was a decrease in posture, justified by the fact that the sick organism going through stress, prioritizes the nutrients absorbed, such

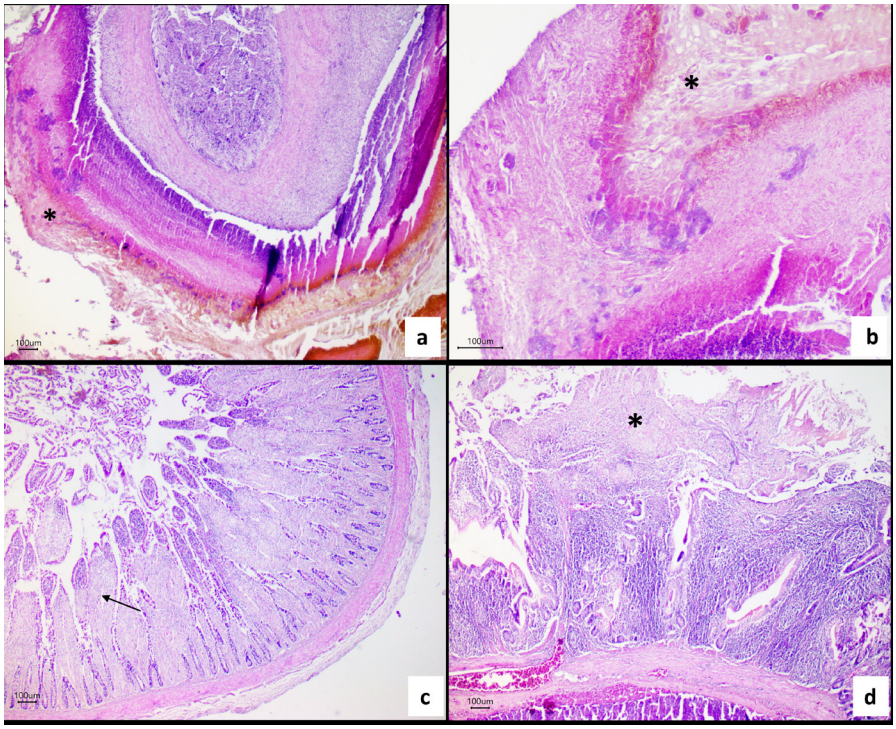

Figure 7. Microscopic findings in the small intestine of Japanese quail. a and b) Celomite causing severe focally extensive necrosis (*) associated with bacterial lumps. Hematoxylin and Eosin, (a) 40x increase and (b) 100x increase. c) Thickened mucosa containing intense multifocal fibrin (arrow). Hematoxylin and Eosin, increase of 40x. d) Severe focally extensive necrotic enteritis (*) in the apical portion of the villi. Hematoxylin and Eosin, 40x increase.

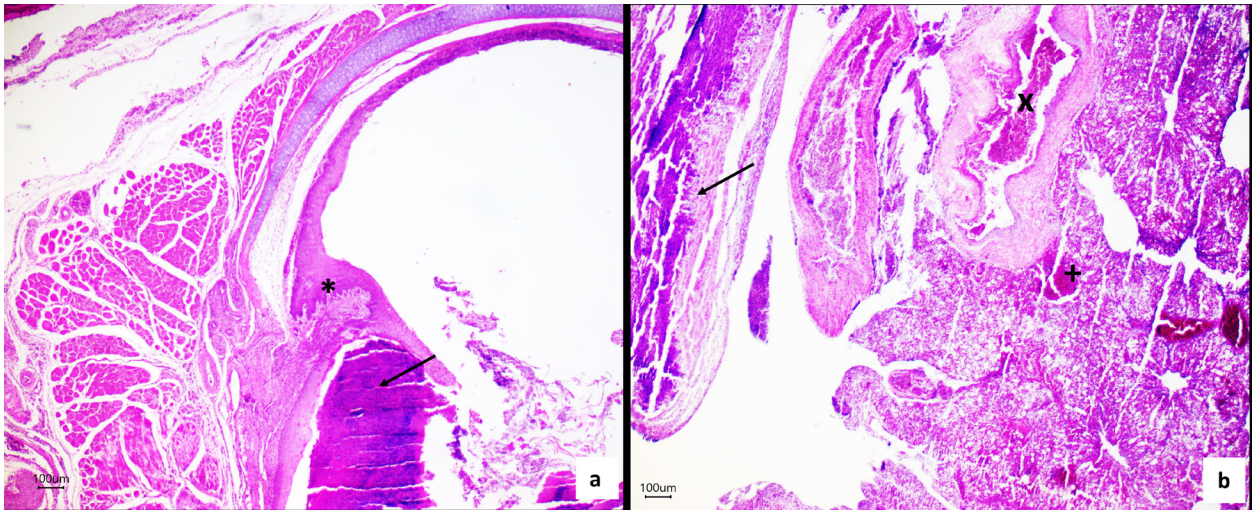

Figure 8. Microscopic findings in trachea and lung of Japanese quail. a) Tracheal mucosa with squamous metaplasia (*) associated with moderate focally extensive necrosis (arrow). Hematoxylin and Eosin, 40x increase. b) Lung with mild multifocal hemorrhage (+), intense multifocal congestion (x), and moderate focally extensive caseous necrosis (arrow). Hematoxylin and Eosin, 40x increase.

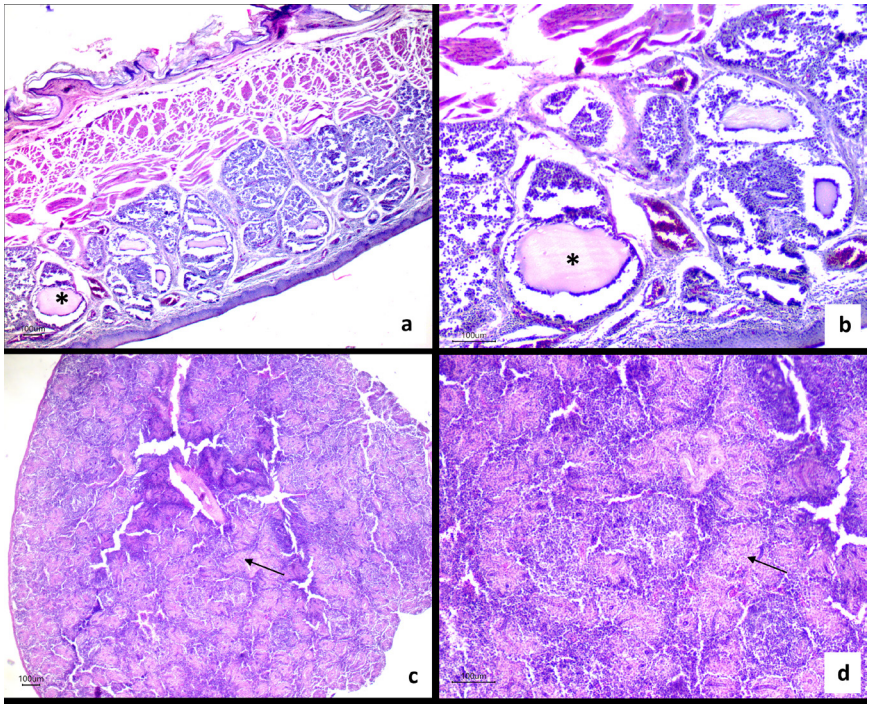

Figure 9. Microscopic findings in the hematopoietic system of Japanese quail. a and b) bursa of Fabricius with moderate multifocal lymphoid rarefaction associated with mild multifocal interstitial edema (*). Hematoxylin and Eosin, (a) 40x increase and (b) 100x increase. c and d) Spleen with intense multifocal white pulp hyperplasia (arrow). Hematoxylin and Eosin, (c) 40x increase. (d) 100x increase.

as calcium, to maintain the homeostasis of the organism, leaving oviposition in the background (Sobreira et al. 2011), as observed in the present study.

However, the findings observed in the bones of the birds of the present study reveal little or no interference of bacterial diseases on the bone tissue of these birds, as well as the survival mechanism generated by these diseases did not significantly interfere in the bone tissue to the level of causing osteopenic diseases, such as osteoporosis, rickets and tibiotarsal dyschondroplasia. This can be justified by the degree of disease manifestation in the body of

these birds, not being enough to reach the bone significantly, considering that the bone tissue of laying birds naturally has more porous bone than compact and the calcium of the compact bone, in cases of malnutrition, is reabsorbed for oviposition (Silva 2020, Konell et al. 2018).

It can also be explained by the fact that the bone is in constant remodeling, being absorbed and formed according to the nutritional needs of layers (Souza et al. 2017). In general, it is important to emphasize that the results of the present study are relevant and considered unprecedented, being of great importance for poultry farming in the State of Espírito Santo as well as for Brazil.

CONCLUSION

It is concluded, therefore, that there was a predominance of alterations in the digestive system and mild parasitic infection. Most of the macroscopic and histopathological lesions were compatible with those caused by the infection of the bacteria found in the microbiological tests, then, the occurrence of colibacillosis and salmonellosis was suggested. However, there was no interference of these anatomopathological changes in the bone tissue in quail.

Acknowledgments

This work was supported by resources from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

REFERENCES

ABPA - ASSOCIAÇÃO BRASILEIRA DE PROTEÍNA ANIMAL. 2021. Relatório Anual. Download from: <https://abpa-br.org/area-exclusiva/abpa-lanca-relatorio-anual-2021/>. Accessed 4 JAN 2023.

ALMEIDA TJO, SANTOS FILHO AA, MACIEL CEM & ROLIM MBQ. 2020. Lesões macroscópicas em órgãos e carcaças de poedeiras comerciais do Agreste de Pernambuco, Brazil. Rev Sem Visu 8: 491-500.

BARNES HJ, NOLAN LK & VAILLANCOURT JP. 2008. Colibacillosis. In: FADLY AM, GLISSON JR, MCDOUGALD LR, NOLAN LK & SWAYNE DE (Eds), Diseases of Poultry, 12. ed, Ames: Blackwell Publishing, p. 691-737.

BENITES MI, STANQUEVIS CE, GRIESER DO, PERINE TP, FINCO EM, MARTINS IO, LIPORI HM & MARCATO SM. 2020. Parâmetros ósseos de codornas de corte suplementadas com diferentes níveis de vitamina A, de 15 a 35 dias de idade. Arq Bras Med Vet Zootec 72: 1497-1503.

CASAGRANDE RA, MACHADO G, GUERRA PR, CASTRO LA, SPANAMBERG A, SILVA SC, CARDOSO MRI & DRIEMEIER D. 2017. Caracterização anatomopatológica e bacteriológica em frangos de corte condenados totalmente por colibacilose sob Serviço de Inspeção Federal. Pesq Vet Bras 37: 949-957.

FERREIRA AJP & KNOBL T. 2020. Colibacilose aviária. In: ANDREATTI FILHO RL, BERCHIERI JÚNIOR A, SILVA EN, BACK A, DI FABIO J & ZUANAZE MAF. Doenças das aves. 3. ed. Campinas: FACTA, p. 519-536.

FREITAS ER, FARIAS NNP, NASCIMENTO GAJ, RODRIGUES AM, QUEIROZ MGR, RAQUEL DL & LIMA RC. 2013. Parâmetros sanguíneos e ósseos de codornas de corte alimentadas com diferentes níveis de sódio. Semin Cienc Agrar 34: 845-852.

FREITAS NETO OC, PENHA FILHO RAC & BERCHIERI JUNIOR A. 2020. Salmoneloses aviárias. In: ANDREATTI FILHO RL, BERCHIERI JÚNIOR A, SILVA EN, BACK A, DI FABIO J & ZUANAZE MAF. Doenças das aves. 3. ed. Campinas: FACTA, p. 491-518.

GOUVEIA ABVS, PAULO LM, SILVA JMS, SILVA WJ, SOUSA FE, JÚNIOR EMA, COSTA TF, SAMPAIO AS, SANTOS FR & MINAFRA CS. 2020. Biometrics of Japanese quails' tíbia and femur fed increasing levels of extruded soybeans. Res Soc Dev 9: e199922249.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA ESTATÍSTICA. 2019. Pesquisa da Pecuária Municipal 2019. Produção da Pecuária Municipal, Rio de Janeiro 47: 1-8. Download from: < https://biblioteca.ibge.gov.br/visualizacao/periodicos/84/ppm_2019_v47_br_informativo.pdf >. Acessed 9 JUN 2022.

ITO NMK & MIYAJI CI. 2020. Fisiopatologia do sistema digestório e anexos dos *Galliformes*. In: ANDREATTI SON RL, BERCHIERI JUNIOR A, SMITH EN, BACK A, DI FABIO J & ZUANAZE MAF. Doenças das aves. 3. ed. Campinas: FACTA, p. 293-240.

KONELL AL, LIMA TR, MARTINI R, LANGE RR & SIERAKOWISKI SS. 2018. Desenvolvimento ósseo e densitometria radiográfica em codorna-japonesa (*Coturnix japonica*). Cienc Anim Bras 19: 1-11.

LEÃO PA, OLIVEIRA LB, ANDRADE MC, HERGOT IG, XAVIER RGC, LEAL CA, DUARTE SC & ECCO R. 2018. Salmonelose tífica em aves de subsistência e de postura comercial: relato de três surtos com ênfase no diagnóstico, epidemiologia e controle. Avicult Industr 109: 14-22.

LOPES MC. 2021. Alterações hepáticas em aves domésticas (Gallus gallus domesticus): estudo retrospectivo e prospectivo de 300 casos. 121f. Dissertação (Mestre em Ciência Animal) – Universidade Federal de Minas Gerais. (Unpublished).

MARTINS NRS, SANTOS RL, COELHO SG, MARQUES JUNIOR AP & ROCHA BD. 2017. Atlas de patologia macroscópica aves e suínos. Cadernos Técnicos de Veterinária e Zootecnia - nº 86. Belo Horizonte: FEPMVZ Editora, 115 p.

MELO AMC DE, SILVA TC DA, SILVA AGS DA, CARNEIRO RSR, PONTES RC DE, SOUZA HCC DE & ALBUQUERQUE IMB. 2018. Doenças infecciosas das aves: revisão de literatura. Braz J Anim Environ Res 1: 310-314.

NASCIMENTO JG, ZICA AR, PRADO AWS & PASSOS PIB. 2021. Criação de codornas para corte. Brasília: EMATER-DF, 56 p.

NARANJO LFN. 2011 Isolamento e propagação de Astrovírus, Adenovírus, Coronavírus, Parvovírus, Rotavírus e Reovírus de aves comerciais com problemas entéricos em ovos embrionados. 147f. Dissertação (Mestrado em Patologia Experimental e Comparada) – Universidade de São Paulo. (Unpublished).

NOLAN LK, VAILLANCOURT JP, BARBIERI NL & LOGUE CM. 2020. Colibacillosis. In: SWAYNE DE ET AL (Eds), Diseases of Poultry. 14. ed. Iowa: Wiley-Blackwell, p. 770-830.

SANTOS BM, MOREIRA MAS & DIAS CCA. 2008. Manual de Doenças Avícolas. Viçosa: Editora UFV, 224 p.

SEEDOR JG, QUARTUCCIO HA & THOMPSOM DD. 1991. The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. J Bone Miner Res 6: 339-346.

SHEATHER AL. 1923. The detection of intestinal protozoa and mange parasites by a floatation technique. J Pathol Ther 36: 266-275.

SILVA EIC. 2020. Anatomia das aves domésticas - anatomia da galinha. WebArtigos. Online. Download from: <https://www.webartigos.com/artigos/anatomia-das-aves-domesticas-anatomia-da-galinha/166310>. Acessed 29 MAR 2023.

SOBREIRA RR, GUEDES JFH, GUALBERTO J & SILVA JR. 2011. Fisiologia reprodutiva das aves. WebArtigos. Online. Download from: https://www.webartigos.com/artigos/fisiologia-reprodutiva-das-aves/64373>. Accessed: 30 MAR 2023.

SOUZA AFGO. 2012. Tecido ósseo em frangos de corte. Nutritime 9: 1663-1679.

SOUZA CS, BARRETO SLT, VIEITES FM, CALDERANO AA, MORAES GHK & OLIVEIRA MGA. 2017. Cálcio e fósforo na nutrição de codornas japonesas em postura. Sci Anim Health 5: 260-281.

TAUNDE PA. 2021. Caracterização patológica e molecular da colibacilose em frangos de corte de moçambique e em galinhas poedeiras do Brasil. 51f. Tese (Doutorado em Ciências) – Universidade Federal do Rio Grande do Sul. (Unpublished).

How to cite

NEIVA DC ET AL. 2024. Anatomopathological changes in laying quails. Na Acad Bras Cienc 96: e20230815. DOI 10.1590/0001-3765202420230815.

Manuscript received on July 26, 2023;
accepted for publication on February 4, 2024

DÉBORA C. NEIVA[1,4]
https://orcid.org/0000-0003-1816-7370

MATHEUS J. DOS S. CANDIDO[1]
https://orcid.org/0000-0002-9075-1160

AMANDA A. ASSIS[1,4]
https://orcid.org/0009-0004-4889-5621

THAIS G. TAVARES[1,4]
https://orcid.org/0000-0002-3065-8052

MÁRCIO P.A. CORREIA[1,4]
https://orcid.org/0000-0003-1349-5498

EMERSON A. DE O. SOUZA[1]
https://orcid.org/0009-0003-5628-628X

DIRLEI. M. DONATELE[2]
https://orcid.org/0000-0002-5697-749X

IGOR C.K. DA CRUZ[1,4]
https://orcid.org/0000-0002-0615-9990

ISABELLA V.F. MARTINS[1,4]
https://orcid.org/0000-0002-8700-3065

MARIA A. DA SILVA[3,4]
https://orcid.org/0000-0003-0967-3925

JANKERLE N. BOELONI[1,4]
https://orcid.org/0000-0003-0049-6854

[1]Universidade Federal do Espírito Santo/UFES, Hospital Veterinário/HOVET, Centro de Ciências Agrárias e Engenharias/CCAE, Laboratório de Patologia Animal, Km 77, BR 482, Rive, 29500-000 Alegre, ES, Brazil

[2]Universidade Federal do Espírito Santo/UFES, Hospital Veterinário/HOVET, Centro de Ciências Agrárias e Engenharias/CCAE, Laboratório de Microbiologia Veterinária, Km 77, BR 482, Rive, 29500-000 Alegre, ES, Brazil

[3]Universidade Federal do Espírito Santo/UFES, Centro de Ciências Exatas, Naturais e da Saúde (CCENS), Departamento de Biologia, Alto Universitário, s/n, Guararema, 29500-000 Alegre, ES, Brazil

[4]Programa de Pós-Graduação em Ciências Veterinárias/PPGCV, Universidade Federal do Espírito Santo/UFES, Centro de Ciências Agrárias e Engenharias/CCAE, Alto Universitário, s/n, 29500-000 Alegre, ES, Brazil

Correspondence to: **Jankerle Neves Boeloni**
E-mail: jankerle@gmail.com

Author contributions

DCN wrote the project, as well as performed all the methodological procedures to obtain the data. MJSC participated in the necropsy, x-ray and microbiological tests of the birds. AAA performed the necropsy of the birds, microbiological tests and decalcification of the bones. TGT performed the necropsy of the birds and decalcification of the bones. MPAC performed the necropsy of the birds and histological processing. EAOS performed the necropsy of the birds. DMD performed the microbiological diagnosis of the samples. ICKC contributed to the descriptions and diagnoses of the alterations observed during the radiographs. IVFM performed the parasitological diagnosis of the samples The entire project was done under the help and guidance of Jankerle Neves Boeloni, along with the co-orientation of Maria Aparecida da Silva.