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Hepatic changes in *Gallus gallus domesticus* in Brazil[1]

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ABSTRACT.- Lopes M.C., Freitas Neto O.C., Amaral C.I., Lacerda M.S.C., Fonseca C.S., Martins N.R.S. & Ecco R. 2022. **Hepatic changes in *Gallus gallus domesticus* in Brazil.** *Pesquisa Veterinária Brasileira 42:e07078, 2022.* Setor de Patologia Animal, Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Belo Horizonte, MG 31270-901, Brazil. E-mail: ecco@vet.ufmg.br

The occurrence and the frequency of hepatic changes in chickens, including broiler (BC), layer (LC) and backyard chickens (BYC) were investigated. The retrospective and prospective study (2006-2021) was conducted with a total of 300 cases of liver disorders. Industrial poultry (BC and LC) were frequently affected (88%) and noninfectious changes were the most diagnosed (69%). Considering etiology or conditions, the hepatic changes were classified as follows: degenerative changes (42%), bacterial (28%), metabolic (15%), toxic (8%), viral (3%), neoplastic (2%), protozoal diseases (1.5%) and circulatory disorders (0.5%). Regarding the type of bird, degenerative, toxic changes and viral hepatitis were more frequent in BC. Circulatory and metabolic disorders, as well as bacterial hepatitis, were more frequently diagnosed in LC. Neoplastic and protozoal hepatitis occurred more frequently in BYC. The macroscopic examination in association with histopathology enabled the diagnosis of the hepatic changes in 59% of the cases. Considering bacterial hepatitis in commercial poultry, the etiological diagnosis is highly important, in view of the risk for public health, despite the obvious importance due to the productivity losses and condemnation at processing.

INDEX TERMS: Liver diseases, avian diseases, *Gallus gallus domesticus*, macroscopic lesions, histopathology, differential diagnosis.

RESUMO.- [**Alterações hepáticas em *Gallus gallus domesticus* no Brasil.**] A ocorrência e a frequência de alterações hepáticas em aves, incluindo frangos de corte (FC), galinhas poedeiras (GP) e aves de subsistência (AS) foram investigadas. O estudo retrospectivo e prospectivo (2006-2021) foi realizado com um total de 300 casos de alterações hepáticas. Aves industriais (FC e GP) foram frequentemente acometidas (88%) e as alterações não infecciosas foram as mais comumente diagnosticadas (69%). Quanto à etiologia ou condição, as alterações hepáticas foram classificadas da seguinte forma: alterações degenerativas (42%), bacterianas (28%), metabólicas (15%), tóxicas (8%), virais (3%), neoplásicas (2%), bem como doenças por protozoários (1,5%) e distúrbios circulatórios (0,5%). Em relação ao tipo de ave, alterações degenerativas, tóxicas e hepatites virais foram mais frequentes nos FC. Distúrbios circulatórios e metabólicos, assim como hepatites bacterianas, foram diagnosticados com maior frequência nas GP. Neoplasias e hepatite por protozoário ocorreram com maior frequência em AS. O exame macroscópico associado à histopatologia possibilitou o diagnóstico da alteração hepática em 59% dos casos. Considerando a hepatite bacteriana em aves comerciais, o diagnóstico etiológico é de grande importância, em vista do risco para a saúde pública, apesar da óbvia importância devido às perdas de produtividade e condenação no processamento.

TERMOS DE INDEXAÇÃO: Alterações hepáticas, doenças hepáticas, doenças aviárias, *Gallus gallus domesticus*, lesões macroscópicas, histopatologia, diagnóstico diferencial.

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INTRODUCTION

The avian liver can be affected by multiple conditions or etiologies. Among the non-infectious causes, there are degenerative changes, such as hydropic, glycogenic and fatty degeneration; metabolic disorders such as hemosiderosis (Abdul-Aziz & Fletcher 2016), amyloidosis (Murakami et al. 2013b, Ibi et al. 2015, Carnaccini et al. 2016), urate deposition (Crespo & Shivaprasad 2013), fatty liver and hemorrhagic liver syndrome (Mete et al. 2013, Trott et al. 2014), as well as circulatory disorders (Abdul-Aziz & Fletcher 2016), and primary neoplasms such as hepatocellular adenoma, hepatocellular carcinoma, cholangioma and cholangiocarcinoma (Abdul-Aziz & Fletcher 2016, Williams et al. 2020). In addition to these, the liver is also affected by neoplasms of infectious etiology, such as in Marek's disease (MD), lymphoid leukosis and reticuloendotheliosis viruses (Abdul-Aziz & Fletcher 2016).

Among bacterial causes, *Salmonella enterica* (Rezende et al. 2008), *Escherichia coli* (Barcelos et al. 2006, Casagrande et al. 2017), *Staphylococcus* spp. (Barcelos et al. 2006, Casagrande et al. 2017, Meyer et al. 2021), *Streptococcus* spp. (Hess et al. 2020), *Clostridium perfringens* (Barcelos et al. 2006), *Pasteurella multocida* (Saif 2013), *Erysipelothrix rhusiopathiae* (Bricker & Saif 2013), *Trueperella pyogenes* (Silva et al. 2020) and *Listeria monocytogenes* (Crespo et al. 2013) were documented. Regarding viral agents, these include avian adenovirus (Sun et al. 2019a), avian hepatitis E virus (Sun et al. 2019b), avian influenza virus (Swayne et al. 2013) and Newcastle disease virus (Miller & Koch 2013). Regarding parasitic agents, *Histomonas meleagridis* is a frequently reported etiology (Araújo et al. 2015).

Considering bacterial hepatitis, the diagnosis is based on macroscopy, histopathology, isolation and characterization (Barcelos et al. 2006, Casagrande et al. 2017). For the diagnosis of viral etiologies, in addition to histopathology, virus isolation and ancillary tests such as PCR (polymerase chain reaction) are used (Sun et al. 2019a). For protozoan hepatitis, such as histomoniasis, the macroscopic evaluation, is important, and histopathology should be used to identify trophozoites (Araújo et al. 2015).

Despite of the variety of etiologies involved in liver pathology and their impact on poultry farming, surveys that exclusively address liver diseases are rarely documented. Some studies involving causes of mortality in chicken (*Gallus gallus domesticus*) have been carried out in Canada (Brochu et al. 2019), United States (Mete et al. 2013, Crespo & Senties-Cue 2015, Cadmus et al. 2019), Finland (Pohjola et al. 2015), Denmark (Stokholm et al. 2010, Thøfner et al. 2019), Sweden (Fossum et al. 2009) and Switzerland (Kaufmann-Bart & Hoop 2009). However, studies involving liver disease and affecting associated anatomical systems are scarce, especially in Brazil.

The aim of this study was to determine the occurrence, types and frequency of chicken liver changes, mainly addressing macroscopic, histopathological and etiological findings.

MATERIALS AND METHODS

Samples. Industrial broiler (farms and slaughterhouses), layer and backyard chickens were included in this study, all of which with morbidity and submitted to *post-mortem* examination and/or histopathological analyses, among other tests, as performed at the "Setor de Patologia Animal" (Veterinary Pathology Sector) of the "Departamento de Clínica e Cirurgia Veterinárias" (Department of Clinical and Veterinary Surgery) and in the "Laboratório de Doença das Aves" (Avian Diseases Laboratory) of the "Departamento de Medicina Veterinária Preventiva" (Department of Preventive Veterinary Medicine), of the "Escola de Veterinária" (School of Veterinary) at the "Universidade Federal de Minas Gerais" (UFMG). The study was retrospective and prospective, and the sampling period comprised from January 2006 to January 2021 (15 years). Two hundred forty-three chickens (n=243) were included, of which 28 had two distinct diagnoses. In addition, 29 cases referring to "pool" of livers and other tissues were included; each "pool" was counted as one case/bird, thus totaling 272 cases with 300 diagnoses (Table 1).

Data collection and history. The anatomical and histopathological data were retrieved from the archives of the Laboratory of Veterinary Pathology, together with the details referring to each chicken or broiler. The diagnoses of liver disease or alteration was obtained at the routine necropsy and histopathology, as well as diagnoses confirmed by other auxiliary tests.

Gross and histopathology. Necropsies were performed following the standard technique for the species. During the necropsy, a macroscopic evaluation of the organs was performed, with a detailed description of the lesions and collection of samples for histopathology. In many cases, necropsies were performed on the farms/properties, with samples sent by the farm veterinarians for the purpose of histopathological diagnosis (samples fixed in 10% formalin) and, in some cases, refrigerated samples for bacteriology.

When necessary, euthanasia was performed by cervical dislocation according to the guidelines on euthanasia Resolution No. 1000/2012 of "Conselho Federal de Medicina Veterinária" (Federal Council of the Veterinary Medicine – CFMV) (CFMV 2012).

For histopathology, tissues fixed in 10% neutral formalin were submitted to the routine histological processing technique (Prophet et al. 1992). Subsequently, the tissues were embedded in paraffin and sectioned in a microtome at 4.0μm of thickness, stained with hematoxylin and eosin (HE) and examined under a standard microscope. When possible, ancillary tests were performed, as indicated after macroscopic and/or histopathological examination. The examinations included special stains, immunohistochemistry, bacteriology, virology, PCR and transmission electron microscopy (TEM).

Histochemical stains. Special staining by periodic acid-Schiff (PAS), Congo red, Prussian blue (Perls) and Masson's Trichrome (Prophet et al. 1992) were performed in livers with macroscopic lesions and histologic findings indicative of glycogen degeneration, protozoan hepatitis, metabolic changes such as amyloidosis and hemosiderosis, and chronic lesions (fibrosis), respectively. In cases which the Congo red stain was performed, the samples were analyzed using polarized light microscopy.

Table 1. Broiler, layer and backyard chicken liver samples diagnosed with hepatic lesions

Type of chickens	Number of chickens	Number of chickens with two diagnoses	Pools	Total of diagnoses
BC	124	13	23	160
LC	89	9	5	103
BYC	30	6	1	37
Total	243	28	29	300

BC = broiler chickens, LC = layer chickens, BYC = backyard chickens.

Bacteriology. Samples from livers and occasionally other tissues (spleen and/or pectoral muscle) from birds with bacterial infection, obtained during necropsy or received under refrigeration, were aseptically sampled for bacteriology in laminar flow cabinets. All specimens were swabbed and plated on tryptic soy agar (Kasvi), containing 5% of sheep blood and in MacConkey agar (Kasvi) which were incubated at 37°C for 24 h (Braga et al. 2016). Finally, the obtained bacterial colonies were identified by the Microflex MALDI Biotyper equipment (Company BD/Bruker, Fremont/CA, USA). Isolation of *Salmonella* spp. from tissue samples followed by the protocol recommended by the "*Ministério da Agricultura*, *Pecuária e Abastecimento*" (Brazilian Ministry of Agriculture, Livestock and Food Supply – MAPA) (Brasil 1995). Aseptically collected fragments of liver and spleen (2g) were placed in sterile tubes with 18mL of brain infusion broth (OXOID) which were incubated at 37°C for 24 h. After that, samples were plated on MacConkey (OXOID) and brilliant green (OXOID) agar plates which were incubated overnight at 37°C. Colonies with aspects suggestive of *Salmonella* spp. were sampled and stained by Gram, selected and submitted to preliminary biochemical screening onto triple sugar agar (TSI), lysine iron agar (LIA), sulfide-indole-motility medium (SIM) and urea. Those colonies with biochemical profiles compatible with *Salmonella* spp. were subcultured on lysogen agar (OXOID) and then tested with polyvalent somatic (O) and flagellar (H) antisera (Probac). Colonies with characteristics (small, no motility, serologically negative for flagellar antigens and poor H_2S production) of *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovars Pullorum or Gallinarum (*Salmonella* Pullorum or *Salmonella* Gallinarum) were submitted to genotyping using duplex PCR for the identification and differentiation between these biovars (Batista et al. 2016).

DNA extraction and PCR. Chromosomal DNA was extracted of the *Salmonella* isolated colonies, as described previously (Marmur 1961), and then submitted to a previously described duplex-PCR reaction (Batista et al. 2016). The *Salmonella enterica* subspecies *enterica* serovar Gallinarum was identified by the identifier region (SIR) and the differentiation between biovars Gallinarum and Pullorum was achieved through the demonstration of polymorphisms of the *rat*A gene. *Salmonella* Pullorum strain ATCC 9120, *Salmonella* Gallinarum strain ATCC 9184 and *Escherichia coli* strain ATCC 25922 were used as control.

Liver from broiler breeds with histologic lesions of adenovirus were used for total DNA extraction. The technique used was based on 6M sodium iodide (NaI) (Vogelstein & Gillespie 1979, Boom et al. 1990) and silicon dioxide (Boom et al. 1990). Subsequently, the total DNA of each sample was transferred into a sterile microtube and stored at -20°C. The PCR was performed to amplify the avian adenovirus hexon gene L1 loop region gene. The extracted DNA was submitted to PCR using reaction conditions and oligonucleotides for *Aviadenovirus* (Meulemans et al. 2001). As positive control, the strain Phelps (kindly provided by Dr. J,K.A. Cook) was used. For the negative control, the DNA was replaced by ultra-pure water.

Transmission electron microscopy (TEM). Livers (n=7) available fixed in buffered formalin, and previously diagnosed with marked amyloidosis by histopathology and histochemistry, were sectioned into smaller fragments, washed in distilled water and post-fixed in glutaraldehyde diluted in PBS buffer solution for 24 hours. Then, the samples were washed, dehydrated with a series of graded alcohols, introduced into resin, cut into ultra-thin sections, placed on copper grids and contrasted, as described previously (Nakayama et al. 2017). The ultra-thin sections were analyzed in a transmission electron microscope at the Center of Microscopy at the UFMG and the images were obtained digitally.

RESULTS

Categorization of chickens examined in this study

Of the 272 chickens (243 individual chickens and a pool of 29 chickens) diagnosed with hepatic changes, 147 (54%) were broiler chickens (BC) (including five broiler breeders), 94 (35%) were layer chickens (LC) and 31 (11%) were backyard chickens (BYC) (Table 1). The age of examined chickens ranged from 1 day-old to 109-week-old. In 52 chickens the age was not specified in the submission forms. Most chickens belonged to farms from state of Minas Gerais (236 in total; 87%). The other chickens were from states of Rio de Janeiro, Espírito Santo and Distrito Federal.

Diagnoses

The hepatic changes were identified as provoked by infectious or noninfectious causes (Table 2). The noninfectious causes were more frequent and comprised 68% of the cases. The most frequent hepatic changes were degenerative lesions (42%) followed by bacterial diseases (28%), metabolic (15%), toxic (8%), viral (3%), neoplastic (2%), protozoal (1.5%), and circulatory changes (0.5%).

Most of samples (79%) were submitted for histopathology only, and no complementary microbiological tests were possible to perform. The macroscopic and histopathological findings enabled the definitive etiological diagnosis in 59% of the chickens. In 25.5% of chickens, ancillary tests were needed for the final diagnosis or to define the etiology. In 15.5% of chickens, the histopathological findings were insufficient, or it was not possible to carry out complementary tests to determine the etiology. For chickens with an indication and possibility for ancillary tests, the results are listed in Table 3.

Hepatic changes frequency on the different types of chickens

Of the 147 broilers, five were broiler breeders (chicks) and in 13 individuals there were two distinct concomitant diagnoses, comprising a total of 160 diagnoses. In these chickens, degenerative changes were more frequent (61%), followed by bacterial (22%), toxic (12%) and viral diseases (5%). The age of these chickens ranged from 1- to 45- day-old.

Of the 94-layer chickens examined in this study, nine had two different diagnoses, thus comprising a total of 103 causes of morbidity. In these chickens, bacterial diseases were more frequent (40%), followed by metabolic (33%), degenerative (19%) and toxic diseases (5%), and finally by circulatory disturbance (2%) and neoplastic diseases (1%). The age of these chickens ranged from 4-day-old to 109-week-old.

Of the 31 backyard chickens, two distinct diagnoses were obtained in six chickens, totalizing 37 different diagnoses. The most frequent changes in these chickens were metabolic diseases (32.5%) followed by degenerative (24%), bacterial (19%), neoplastic (13.5%) and protozoal diseases (11%). The affected ages ranged from 4- to 104-week-old.

Clinical history, gross and histologic lesions of degenerative hepatic changes

Degenerative hepatic changes were diagnosed in 98 broilers (4 to 45 days old), of which 63 (63/98) were related

to lipidosis and 35 (35/98) to glycogen degeneration (4 to 40-day-old). Of the 63 broilers diagnosed with lipidosis, 35 (35/63) originated from livers collected at processing plants, as discarded by the sanitary inspection service. The remaining cases of lipidosis coincided with diseases in other anatomic systems, as the respiratory system. Of the 35 cases of glycogen degeneration, five (5/35) comprised livers discarded at broiler processing.

Table 2. Diagnoses of hepatic changes in backyard, broiler and layer chicken categorized by etiologic or condition classification

Noninfectious changes and diseases					
	Diagnoses	Chickens			Total
		BC	LC	BYC	
Degenerative	Lipidosis	63	14	7	84
	Glycogen degeneration	35	6	2	43
n/%		98/77	20/16	9/7	127/100
General degenerative/general total/%				127/300/42%	
Metabolic	Amyloidosis	0	29	3	32
	Hemosiderosis	0	2	6	8
	Hepatic urate deposition	0	0	3	3
	Fatty liver hemorrhagic syndrome	0	3	0	3
n/%		0/0	34/74	12/26	46/100
General metabolic/general total/%				46/300/15%	
Toxicoses	Toxic hepatopathy	19	5	0	24
n/%		19/79	5/21	0/0	24/100
General toxicoses/general total/%				24/300/8%	
Circulatory disturbances	Cardiac liver	0	2	0	2
n/%		0/0	2/100	0/0	2/100
General circulatory disturbances/general total/%				2/300/0.5%	
Neoplastic	Lymphoid leukosis	0	1	2	3
	Marek's disease	0	0	2	2
	Hepatocellular adenoma	0	0	1	1
n/%		0/0	1/17	5/83	6/100
Total		117	62	26	205
Infectious diseases					
Bacterial	Undetermined	25	18	3	46
	Salmonellosis	0	14	3	17
	Colibacillosis	3	5	1	9
	Streptococosis	7	4	0	11
n/%		35/42	41/49	7/9	83/100
General bacterial/general total/%				83/300/28%	
Viral	Inclusion body hepatitis*	5	0	0	5
	Undetermined	3	0	0	3
n/%		8/100	0/0	0/0	8/100
General viral/general total/%				8/300/3%	
Protozoal	Histomoniasis	0	0	4	4
n/%		0/0	0/0	4/100	4/100
General protozoal/general total/%				4/300/1.5%	
TOTAL		43	41	11	95
GENERAL TOTAL/%		160/53	103/34,5	37/12,5	300/100

n = number of chickens, BC = broiler chickens, LC = layer chickens, BYC= backyard chickens; * Broiler breeder.

In layer hens, hepatic degeneration was diagnosed in 20 chickens, of which 14 (14/20) had lipidosis. The ages were one-week (physiological lipidosis), 46-, 86- and 109-week-old. Chickens diagnosed with glycogen degeneration were 30-week-old (5/6) and 109-week-old (1/6). In layer hens with lipidosis, mild reductions in the quality and quantity of eggs produced were reported, in addition to a slight increase in the mortality rate.

In backyard chickens, the degenerative hepatic changes comprised nine cases, seven (7/9) of which had lipidosis and two (2/9) had glycogen degeneration. The highest age of 104 weeks was recorded for one chicken. All the chickens

Table 3. Type of chicken, ancillary tests and diagnosis of liver changes or diseases in commercial and backyard chickens analyzed from 2006 to 2021

Type of chickens	Number	Ancillary tests and etiology	Diseases/Condition
		Bacteriology	
Broiler	3	*Escherichia coli*	Colibacillosis
	7	*Streptococcus gallolyticus*	Streptococcosis
Layer	14	*Salmonella* spp.	Salmonellosis
	4	*Streptococcus gallinarum*	Streptococosis
	5	*Escherichia coli*	Colibacillosis
Backyard	1	*Escherichia coli*	Colibacillosis
	3	*Salmonella* spp.	Salmonellosis
Total	37		
		Histochemical = PAS	
Broiler	26	Glycogen	Glycogen degeneration
Backyard	4	*Histomonas meleagridis*	Histomoniasis
Total	30		
		Histochemical = Congo red	
Layer	29	AA amyloid	Amyloidosis
Total	29		
		Histochemical = Prussian blue	
Layer	2	Hemosiderin	Hemosiderosis
Total	2		
		Histochemical = Masson's trichrome	
Layer	2	Fibroplasia	Chronic hepatopathy
Total	2		
		PCR	
Broiler*	3	*Aviadenovirus*	Inclusion body hepatitis
Layer	10	*Salmonella* Gallinarum	Fowl typhoid
Backyard	3	*Salmonella* Gallinarum	Fowl typhoid
Total	16		
		Immunohistochemistry**	
Backyard	3	*Salmonella* spp.	Salmonellosis
Total	3		
		Electron microscopy	
Layer	7	AA amyloid	Amyloidosis
Total	7		
OVERALL TOTAL	127		

PAS = periodic acid-Schiff, PCR = polymerase chain reaction, NA = not applicable. In number, the first is the number of results found, the second is the number of birds tested; * Broiler breeder, ** Salmonella polyvalent antibody (Probac do Brasil, São Paulo/SP) was used at a dilution of 1:2000 in the liver tissue for immunohistochemistry (three backyard chickens). Incubation with secondary antibody was performed with EnVision-Dual link (Dako, Carpinteria/CA, USA).

with lipidosis were apathic and some had respiratory disease and emaciation.

Macroscopically, lipidosis-related changes were similar among chickens but with different intensities. In broilers, the livers were yellow-red to diffusely yellow and friable. In layer hens, in addition to yellow discoloration, the livers were oily, enlarged and with abundant celomatic adipose tissue covering the organs. Histologic lesions of lipidosis were characterized by enlarged hepatocytes containing intracytoplasmic well-delimited vacuoles with the nucleus displaced to the periphery. The distribution and intensity ranged from mild to marked in all types of chickens. In cases diagnosed with glycogen degeneration, the livers were pale-red and microscopically there were cytoplasmic small and poorly delimited vacuoles (Fig.1), variable intensity and distribution. Of these cases, 26 were PAS positive -magenta color (Fig.1 inset).

Clinical history, macroscopic and histologic lesions of bacterial hepatic diseases

In broilers, indicative lesions of bacterial hepatitis were seen in 35 birds. In ten (10/35) of these cases the etiology was confirmed by bacteriology. *Streptococcus gallolyticus* was identified in 21-day-old broilers (7/10) and *Escherichia coli* in 11-day-old chicks (3/10). In 25 cases (25/35), the bacteriological test was not possible, and the bacterial agent involved was not defined. The age of broilers ranged from one- to 45-day-old. The clinical signs reported included apathy (5/25), respiratory distress (3/25), dehydration (2/25), and yolk sac infection (3/25). The broiler flock with colibacillosis had a moderate increase in mortality and 16% had uneven growth.

Macroscopically, livers of three broilers (3/7) infected by *S. gallolyticus* had multifocal millimetric white areas and in one broiler (1/7) the liver was dark-red interspersed with green areas. Histologic lesions revealed heterophilic and lymphoplasmacytic peri-hepatitis (3/7) with multifocal to coalescing necrotic and heterophilic hepatitis. Biliary ductal proliferation was present in the liver of two broilers (2/7).

In broilers (3/3) diagnosed with colibacillosis, the livers were enlarged, hyperemic and had diffuse fibrinous peri-hepatitis (Fig.2). In two broilers, there were also fibrinous pericarditis and airsaccullitis. Histologically, there were fibrinous and heterophilic peri-hepatitis associated with numerous gram-negative rods.

The main findings in the livers of broilers with presumptive diagnoses of bacterial hepatitis, without confirmation of bacterial agent, were white foci subcapsular and in the parenchyma (11/25), enlargement (3/25), being two of these dark-red (2/25) and another diffusely green (1/25). The histologic lesions for all cases were characterized by necrotic and fibrinous hepatitis to fibrinous heterophilic and lymphohistiocytic hepatitis. Most of these lesions were associated with numerous intralesional bacteria.

In layers, bacterial hepatitis was diagnosed in 41 chickens and *Salmonella* spp. detected in 14 hens (14/41), as confirmed by bacteriology. The ages of the affected chickens were 8-week-old (6/14), 49-week-old (4/14) and 77-week-old (4/14). PCR enabled the confirmation of *Salmonella* Gallinarum biovar Gallinarum in ten (10/14) chickens. In the other cases (4/14), the *Salmonella* serovar was not confirmed. In 9-week-old layer chickens (5/41), the bacteriology enabled the isolation of *E. coli* and in four 91-week-old layer chickens, *Streptococcus gallinarum* was isolated. In 18 cases (18/41), bacteriology was not possible, and the bacterial agent involved was not defined. The age of chickens ranged from 4-day-old to 109-week-old.

Layer chicken flocks diagnosed with fowl typhoid had increase mortality, ranging from 5 to 10%, reaching up to 80% in one farm. In six (6/14) cases, a drop in egg production was observed, and in four cases (4/14), the chickens showed apathy. In the layer flocks diagnosed with colibacillosis (5/5), chickens had apathy, respiratory distress and the mortality rate reached 2.5%.

Macroscopically, the livers of layers diagnosed with fowl typhoid were enlarged (14/14), diffusely bronze to dark-red and occasionally mottled with multiple white foci (11/14) or green (3/14) (Fig.3). In 12 cases (12/14) the spleen was enlarged, sometimes 2-3 times, and in one case (1/14) there were multiple white areas in the myocardium and epicardium. The hepatic histologic lesions (Fig.4) were characterized by random multifocal necrosis associated with heterophils (10/14), and histiocytes (4/14), in addition to deposition of abundant fibrin in the wall of sinusoids. Lymphohistiocytic myocarditis with loss of cardiomyocytes were seen in the heart.

In layer chickens diagnosed with colibacillosis, there were marked fibrinous peri-hepatitis (4/5) and fibrinous pericarditis (3/5), and enlarged and mottled liver with multiple millimetric white areas of necrosis (1/5). At histopathology, there were fibrinous peri-hepatitis with microthrombi in the sinusoids (5/5) and fibrinonecrotic splenitis.

Layer chickens diagnosed with *Streptococcus gallinarum* had enlarged livers (4/4), with yellow discoloration (2/4), multiple miliary white foci (3/4), or whitish fibrillar material covering the capsular surface (1/4). Histologic lesions revealed lytic necrosis associated with heterophils, fibrin (2/4), in addition to heterophils, lymphocytes and histiocytes in the periportal area and biliary ducts. Adhered to the ductal epithelia there were many gram -positive cocci characterizing a heterophilic and lymphohistiocytic cholangiohepatitis (2/4).

In 18 layers with indicative diagnosis of bacterial hepatitis without confirmation of bacterial agent, gross and histologic lesions were similar to those described above. Livers were enlarged (5/18) with yellow to green discoloration or mottled with multiple white foci of necrosis. At histopathology, multifocal random fibrinonecrotic and heterophilic hepatitis were the most common changes.

In backyard chickens, bacterial hepatitis occurred in seven chickens and *Salmonella* spp. was confirmed by bacteriological examination and immunohistochemistry in three (3/7) 12-week-old chickens. One case (1/7) was associated with *E. coli* and in three chickens the bacterial agent was not determined. Gross and histologic lesions in these chickens were similar to those aforementioned for bacteriologic hepatitis in laying hens.

Clinical history, macroscopic and histologic lesions of metabolic hepatic changes

Metabolic diseases were identified in 34 industrial layer chickens, of which 29 (29/34) were diagnosed as amyloidosis. Out of the amyloidosis cases, 12 were found in 12 weeks old and seven were in 16 weeks old. In addition, three cases (3/34) of fatty and hemorrhagic liver syndrome were diagnosed in 46-week-old chickens and two cases (2/34) of hemosiderosis were diagnosed in 17-week-old laying hens. The other cases

of metabolic changes occurred in 12 backyard chickens: six cases were attributed to hemosiderosis, three cases (3/12) were amyloidosis and the other three cases (3/12) had hepatic deposition of urate crystals.

Amyloidosis of industrial layer chickens (19/29) occurred in a flock with 100,000 chickens. Chickens were vaccinated against Marek's disease, fowlpox, infectious bursal disease, infectious bronchitis, Newcastle disease, *Mycoplasma gallisepticum*, avian pneumovirus, infectious laryngotracheitis (tissue culture origin vaccine - TCO), and inactivated intramuscular (pectoral) vaccination against salmonellosis and infectious coryza. Clinically, chickens showed apathy, drop in egg production, emaciation and increased mortality (0.5 to 4.0%). The main gross findings were enlargement of the liver (8/29) and spleen (12/29). The livers were brownish to pale-red (8/29) (Fig.5). There was atrophy of the pectoral muscles (6/29) and between the fasciae of the pectoral muscles, of the right side, possibly associated to the intramuscular oil-emulsion vaccination, there was hyperemia and multifocal petechiae or white to yellow exudate (7/29). Liver histopathology revealed deposition of amorphous, eosinophilic and homogeneous material in the space of Disse. The deposition resulted in compression with atrophy and multifocal loss of hepatocytes (29/29) (Fig.6). In addition, there were similar depositions in the splenic vessel walls, especially in the ellipsoid area (4/29), and between the muscular fibers of the pectoral musculature, along with lymphoplasmacytic cells associated with loss and fragmentation of myocytes and negative circular images compatible with a mineral oil adjuvanted vaccine. The amyloid deposition in the liver, spleen and pectoral muscles was confirmed by Congo-red special stain and visualization of a bright emerald green color under polarized light. In livers examined by electron microscopy (7/29), deposition and aggregation of non-branched amyloid fibrils, measuring from 7.0 to 11.0nm, were seen in the basal membrane of sinusoids and space of Disse, with compression of adjacent hepatocytes (7/7) (Fig.7). Liver samples from these cases were collected aseptically for bacteriological examination and resulted negative. Amyloidosis was also diagnosed in the liver of three backyard chickens with bacterial infection in other anatomical systems.

Layer chickens (3/3) diagnosed with fatty liver and hemorrhagic liver syndrome had livers enlarged, markedly yellow, hemorrhagic and unctuous. Histological lesions were typical of marked lipidosis associated with hemorrhage.

Hemosiderosis was diagnosed in the liver of backyard chickens (6/8) and layer chickens (2/8). Grossly, the liver of one backyard chicken was yellow-red and with multiple white foci (1/6). The livers of layer chickens were smaller and with millimetric and linear white areas in the parenchyma (2/2). At histopathology, there was granular and brown material inside of the cytoplasm of the hepatocytes as well as in the Kupfer cells in all chickens (8/8). In the livers of the layer chickens, Prussian blue histochemical stain resulted positive, in addition, there were loss of hepatocytes and moderate fibroplasia (2/2).

Deposition of urate in the liver was another metabolic disease identified in three backyard chickens. Chickens were adult and one was identified as 43-week-old. Macroscopically, the surfaces of the livers were covered by deposition of white and rough granular material (Fig.8 inset), as well as on the surfaces of other organs, such as kidney, pericardium, lung and joint. At histopathology, the livers had multiple necrotic areas along with negatively stained and basophilic thin structures radially arranged, typical of urate crystals (Fig.8).

Clinical history, macroscopic and histologic hepatic lesions indicative of toxic changes

Hepatic changes indicative of acute toxicity was found in 19 six to 42 days old broilers. Eleven broilers (11/19) were of four flocks that had history of increased mortality after the exposition to carbamate-based insecticide. Broilers showed decubitus and pedaling movements of the pelvic limbs (2/19) and decreased feed consumption. Grossly, the livers were congested or pale-red to yellow-red. At histopathology, the hepatocytes of centrilobular and midzonal region were swollen and with hydropic degeneration (2/19). In other six broilers (6/19) the hepatocytes of centrilobular and midzonal area had hypereosinophilia, pyknotic and karyorrhexic nuclei. In two cases (2/6) there was a proliferation of biliary ducts.

In layer chickens, liver changes indicative of toxic causes was found in five 17-week-old chickens. Chickens were from the same farm and housed in a place with poor management and showed apathy one to two days before death and had chronic hepatopathy. Grossly, five chickens (5/5) had livers reduced in size and firm, with white and thick capsules, brown parenchyma and dilated gallbladders. There was also hydropericardium. The histopathology findings were characterized by swollen hepatocytes with karyomegaly, predominantly in the centrilobular regions. In addition, there was diffuse hemosiderosis (confirmed by Prussian Blue), mild to moderate fibrosis confirmed by Masson's Trichrome), and bile duct hyperplasia.

Clinical history, macroscopic and histologic hepatic lesions of viral diseases

Hepatic lesions associated with virus were present in eight 21-day-old broiler breeders from two flocks. Of these cases, five (5/8) were attributed to inclusion body hepatitis (IBH). Chickens from these flocks showed decreased growth and an increase in mortality to 1.5% (flock 1) to 4.5 % (flock 2). PCR and sequencing for *Aviadenovirus* confirmed the infection in chickens from flock 1, characterized as fowl adenovirus E, with hepatic necrosis associated with typical intranuclear inclusion bodies in the hepatocytes. Macroscopically, livers were enlarged, diffusely red-yellow and with multiple petechiae (3/5). At histopathology, all chickens had hepatic lipidosis and multifocal random necrotic areas associated with lymphoplasmacytic cells. Basophilic intranuclear inclusions were seen in the hepatocytes, especially in the periphery of necrotic areas (Fig.9). Eosinophilic inclusions surrounded by a clear halo were also observed in some hepatocytes.

Histologic lesions indicative of viral etiology was also found in other cases of hepatitis (3/8) that occurred in 13-day-old broilers; however, an etiologic agent was not confirmed by ancillary tests. The broilers showed clinical signs and increase mortality at 10-day-old. Macroscopically, the livers were yellow and interspersed by hemorrhagic areas. At histopathology, there was necrohemorrhagic and lymphocytic hepatitis.

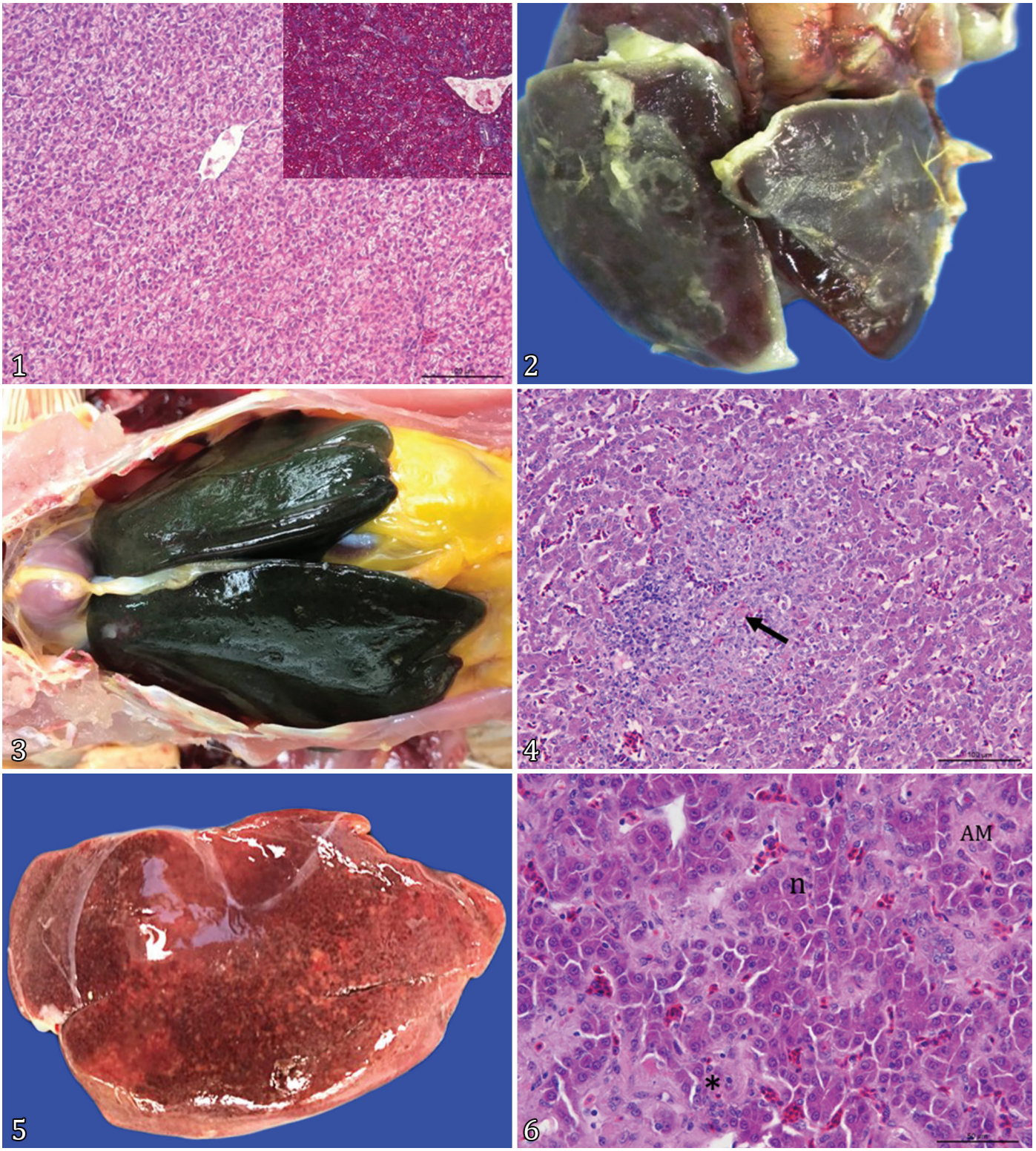

Fig.1-6. Macroscopic and histopathological findings in livers of domestic chickens. (**1**) Twenty-seven-day-old broiler with glycogen degeneration. There is several small and poorly delimited intracytoplasmic vacuolation. HE, obj.10x. Inset: Strong intracytoplasmic magenta stain by periodic acid-Schiff (PAS), confirming glycogen deposition, 200x. (**2**) Eleven-day-old broiler, naturally infected by *Escherichia coli*. Enlarged liver with marked deposition of fibrin on the capsular surface. (**3**) Seventy-seven-week-old laying hen naturally infected by *Salmonella* Gallinarum. Liver enlarged and diffusely dark-green. (**4**) Liver previously shown in Figure 3. Area with loss of hepatocytes and replacement by necrotic cells, inflammatory cells and fibrin (arrow). HE, obj.10x. (**5**) Sixteen-week-old layer chicken with amyloidosis. Enlarged liver with small multifocal to coalescing pale-yellow areas. (**6**) Liver previously shown in the Figure 5. Deposition of amorphous, eosinophilic and homogenous material, compatible with amyloid (AM), in the space of Disse, with compression and loss of adjacent hepatocytes (*). Normal hepatocyte (n). HE, obj.20x.

Clinical history, macroscopic and histologic hepatic lesions of circulatory disturbances

Liver with chronic passive hyperemia due to heart failure (cardiac liver) was diagnosed in two industrial layer chickens, aged 31 and 79 weeks old. These chickens had respiratory distress and cyanosis. Macroscopically, the coelomic cavity was distended, filled by ascitic liquid and the liver was reduced in size, firm and with capsular fibrosis. The heart was enlarged, with ventricular dilatation and thinned myocardium, characterizing dilated cardiomyopathy. In both cases, the histopathology of livers revealed chronic passive hyperemia and centrilobular and bridging fibrosis with loss of hepatocytes.

Clinical history, macroscopic and histologic lesions of hepatic neoplastic diseases

Neoplastic changes were found in one 19-week-old layer chicken and in five adult backyard chickens. Hepatic lymphoid proliferation compatible with lymphoid leukosis was found in layer chicken (1/1) and in backyard chickens (2/5). Macroscopically, the liver of layer chicken was whitish and slightly enlarged and the livers of backyard chickens had multiple white nodules. Neoplastic proliferation in one backyard chicken was seen also in the spleen, ovaries, kidneys and intestine. At histopathology, all chickens had multifocal to coalescing, non-encapsulated and expansive proliferation of monomorphic round cells, morphologically similar to lymphoblasts (lymphoma) in livers, without involvement of nerves.

In backyard chickens, other diagnoses of neoplastic disease were obtained, two associated with Marek's disease (2/5), and one hepatocellular adenoma (1/5). One chicken diagnosed with Marek's disease was 47-week-old. Both chickens diagnosed with Marek's disease had multifocal white nodules in the liver. The nodules ranged from a few millimeters to 1.0cm, were soft and solid. Similar lesions were present in other organs, including the sciatic and brachial nerves. At histopathology, there was an infiltrative proliferation of lymphoid cells markedly pleomorphic with numerous mitoses, replacing the parenchyma of the liver. In the nerves, neoplastic cells infiltration caused axonal loss and degeneration.

Hepatocellular adenoma was characterized macroscopically by hepatomegaly with an expansive tumoral mass of approximately 10cm, friable, pale-red interspersed by dark-red or white areas. At histopathology, neoplastic cells were similar to hepatocytes arranged in cords or acinar formations with or without lumen, supported by a thin connective stroma. There were some necrotic areas, and the adjacent liver parenchyma was compressed and atrophied.

Clinical history, macroscopic and histologic hepatic lesions of protozoal diseases

Histomoniasis was diagnosed in four backyard chickens (4/37), two of which of unknown ages, and the other two were four weeks old. Clinically, these chickens showed apathy, decreased feed consumption and emaciation. In addition to chickens, guinea fowl, geese and ducks coexisted in the same area. Macroscopically, livers of two chickens (2/4) were enlarged, with multiple to coalescing circular, yellow to red areas characterizing necrotic hepatitis (Fig.10). In two chickens (2/4), the livers were yellow-red with multiple white millimetric areas. Histopathological findings were similar in all chickens and characterized by areas of coagulation or lytic necrosis associated with histiocytic to granulomatous inflammation. Along with these areas, there were numerous rounded eosinophilic protozoa measuring 10 to 20μm, with a negative capsule compatible with trophozoites of *Histomonas meleagridis* (Fig.11). PAS stained the trophozoites in the liver of all chickens (Fig.12). Necrotic and histiocytic typhlitis associated with PAS positive trophozoites was also found in two chickens.

DISCUSSION

A variety of hepatic diseases, including of infectious and noninfectious etiologies were found in industrial or free-range chickens, some of which of zoonotic importance.

The macroscopic and histopathological findings enabled the definitive etiological diagnosis in 59% of the chickens. For other cases, ancillary tests were needed for the final diagnosis or to define the etiology, although the lesions were important in defining the type of ancillary test. These results emphasize the importance of the histopathological examination, for the association of the detected etiology with the corresponding lesions (cause and effect), providing a conclusive diagnosis in many avian diseases (Dolka et al. 2012).

Noninfectious hepatic changes were the most frequent in the current study and were represented mainly by degenerative and metabolic changes. Of the degenerative changes, the largest number of cases was associated with lipidosis, occurring mostly in broiler chickens over 30 days old. Several liver samples collected at the processing plant were submitted to histopathology due to suspicion of mycotoxicosis (aflatoxicosis). In a previous study with livers condemned at the veterinary inspection service, some were diagnosed as lipidosis (Barcelos et al. 2006). In the absence of hepatic lesions indicative of toxic causes, as seen in the present study, lipidosis can be related to a high-energy diet (Abdul-Aziz & Fletcher 2016), especially when energy sources are proportionally higher in relation to protein and fiber, and the pre-slaughter period of fasting is shorter than 10-12 hours, time required for the chicken to metabolize and reduce the concentration of fatty acids in the liver (Bartov 1996, Trampel et al. 2005). Furthermore, diet related lipidosis in chickens, may be more common because lipogenesis is greater in the liver, as compared to adipose tissue (Alshamy et al. 2019, Zaefarian et al. 2019). Liver glycogen storage is also an alteration resulting from a high-carbohydrate diet (Trampel et al. 2005, Zaefarian et al. 2019), which induces vacuolar degeneration in the hepatocytes, turning the liver yellowish-red to whitish (Trampel et al. 2005). The decrease in the quantity of glycogen storage in the liver also occurs during pre-slaughter fasting (Warris et al. 1993), a period that contributes to the mobilization of glycogen and lipid reserves, resulting in dark-red color. Liver of chickens that were not submitted to adequate fasting prior to slaughter are whitish or yellow and on the slaughter line they may be interpreted as abnormal and condemned during sanitary inspection (Trampel et al. 2005).

Considering laying hens diagnosed with lipidosis at the peak of production, the hepatic deposition may associate to high energy diets, which may predispose to hepatic lipidosis (Leeson 2007). During egg production, lipogenesis in the liver becomes elevated due to the high level of estrogen (Hermier

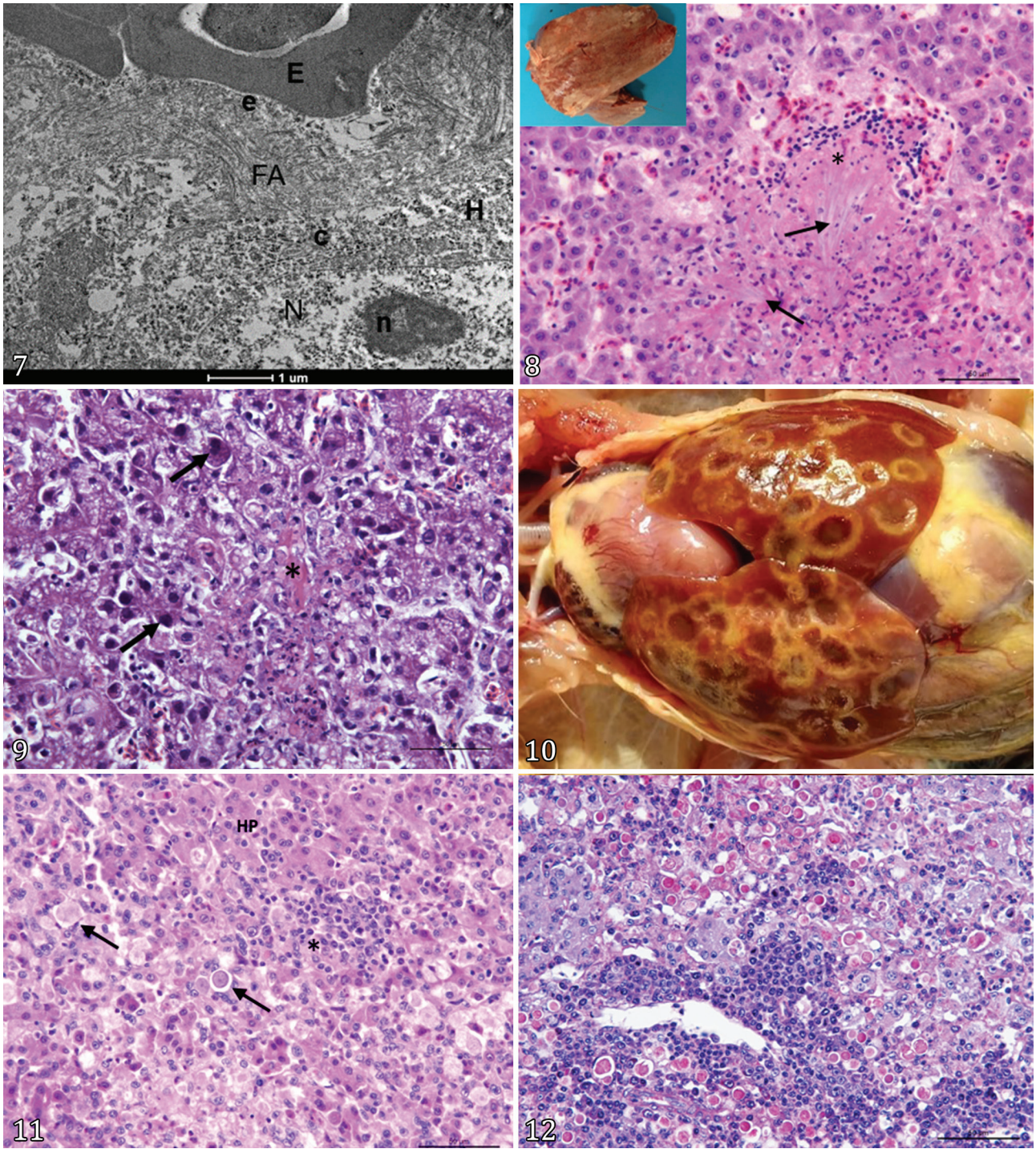

Fig.7-12. Macroscopic, histopathological and electron microscopy findings in livers of domestic chickens. (**7**) Liver previously shown in the Figure 6. Deposition and aggregation of unbranched amyloid fibrils (AF), measuring 7.0 to 11.0nm, in the wall of the sinusoids and space of Disse, compressing adjacent hepatocytes (Hp). Erythrocyte (E); cytoplasm of endothelium (e); cytoplasm of hepatocyte (c); nucleus (N), and nucleolus (n) of hepatocyte. Transmission electron microscopy (TEM). Bar = 1µm. (**8**) Backyard chicken with visceral urate deposition. Area of necrosis with cellular debris and radially arranged negatively stained crystals, characterizing multifocal urate deposition (arrow) associated with granulomatous infiltrate (*). HE, obj.20x. Inset. Enlarged liver with deposition of granular and white material (urate) on the capsular surface. (**9**) Liver. Twenty-one-day-old broiler breeder. Fibrinonecrotic hepatitis (*) and hepatocytes with basophilic intranuclear inclusion bodies filling the entire nucleus and dislocating the chromatin to the periphery (arrow). HE, obj. 40x. (**10**) Four weeks-old backyard chicken naturally infected by *Histomonas meleagridis*. Mildly enlarged liver with an irregular subcapsular surface, characterized by circular well defined centrally red and depressed areas. (**11**) Liver previously shown in the Figure 10. Focally extensive area of necrosis adjacent to preserved hepatocytes (H), associated with lymphohistiocytic and heterophilic infiltrate (*) and numerous rounded eosinophilic protozoa of 10 to 20µm compatible with *Histomonas* trophozoites (arrows). HE, obj.10x. (**12**) Liver previously shown in the Figure 11. PAS-positive *Histomonas meleagridis* trophozoites. PAS, obj.20x.

1997). Furthermore, lack of exercise, due to restricted cage space in combination with high energy diet was also related to hepatic lipidosis (Zaefarian et al. 2019, Klasing & Korver 2020).

Fatty and hemorrhagic liver syndrome was diagnosed in only three layers. The disease has been described as a cause of mortality in industrial layer chickens (Shini et al. 2019) and in backyard chickens (Mete et al. 2013). Factors such as high energy diets, as well as exercise restriction and high temperature may be related to its occurrence in these chickens (Crespo & Shivaprasad 2013, Crespo & Senties-Cue 2015).

Amyloidosis was the most frequent metabolic disease in this study, mostly in laying hens. In 19 cases, the disease was attributed to the type and frequency of vaccines administered to these chickens, including intramuscular vaccines against salmonellosis and infectious coryza. The association with vaccination may be justified by the identification of amyloid deposits in the pectoral muscles (site of vaccine application). Recently, outbreaks of amyloidosis have been reported in laying hens (Murakami et al. 2013b, Ibi et al. 2015, Carnaccini et al. 2016) and in broiler breeders (Tracy et al. 2021) and related to the administration of commercial or autogenous vaccines against salmonellosis (Murakami et al. 2013a, Tracy et al. 2021). In a previous study, amyloidosis that investigated concerning the potential association with administration of adjuvanted vaccines and found that a single company provided commercial vaccines that were used in different farms, which had chickens with amyloidosis (Tracy et al. 2021). Amyloid deposition may have been induced indirectly by persistent inflammatory stimulation at the vaccination site and/or by absorption of the antigens into the circulation (Carnaccini et al. 2016).

As observed in the chickens of the present study, flocks affected by amyloidosis had often increased mortality (Murakami et al. 2013b, Carnaccini et al. 2016, Tracy et al. 2021), due to the severity of liver lesions (Ibi et al. 2015). The three cases in backyard chickens had also chronic inflammatory changes, which may be related to amyloidosis, since chronic diseases also promote hyperstimulation of the immune system (Landman et al. 1998).

Amyloidosis on industrial layer chickens in the current study occurred in white leghorn (Lohmann), as in other studies (Carnaccini et al. 2016, Habibi et al. 2017). Nevertheless, experimental studies involving this, and other breeds of chickens may be needed to clarify the possibility of genetic predisposition.

Hemosiderosis was diagnosed in eight chickens in the present study, and of these, six were backyard chickens and two were laying hens. In chickens, hepatic hemosiderosis seems to be more frequent in birds living in captivity for several years (Wadsworth et al. 1983), and this may explain the few cases reported here. In birds, iron is commonly found in the liver, and can be a concomitant finding in infectious and non-infectious diseases (Cork et al. 1995), as in cases of lead poisoning (Sobhakumari et al. 2018).Urate deposition in the liver occurred in three backyard chickens. Besides the liver, depositions of urate were also visualized in other tissues of these chickens. In this condition, multiple organ involvement is frequent (Mir et al. 2005, Crespo & Shivaprasad 2013), and can be caused by nutritional, metabolic, infectious, and other factors (Crespo & Shivaprasad 2013). In the current study, water restriction or dietary imbalance (excessive protein) are suggested as determining factors. Unlike industrial chickens, in some situations, backyard chickens do not receive a balanced diet. Outbreaks on this condition attributed to dietary imbalance have been reported previously (Mir et al. 2005).

Hepatic changes related to toxic causes in broilers were mainly acute and were indicative of mycotoxin intoxication. In fumonisin B1 intoxication, multifocal liver necrosis and biliary hyperplasia were previously reported (Ledoux et al. 1992, Shlosberg 2008), and it could be included in the list of differentials diagnoses along with aflatoxin and ochratoxin. Analysis of the feed supplied to these chickens would contribute to the definitive diagnosis in these cases (Hoerr 2020).

In a flock of industrial layers of the current study, the chronic lesions involving the liver were indicative of chronic mycotoxin poisoning, possibly aflatoxin. In this intoxication, the liver is often smaller and firm. Histologic lesions are characterized by lipidosis, loss of hepatocytes, fibrosis and biliary hyperplasia (Abdul-Aziz & Fletcher 2016, Hoerr 2020). In addition, in chronic cases, hydropericardium and degenerative kidney lesions may occur (Hoerr 2020), which were also described in the chickens of this study. Other causes to be included in the differential diagnosis are toxic plants containing pyrrolizidine alkaloids, particularly seeds of *Crotalaria* sp. (Pereira et al. 2011). However, no evidence of rattlepods seeds was found in the diet of these chickens. Neoplastic diseases were infrequent in the present study, possibly due to the smaller numbers of long-lived chickens, particularly backyard chickens. Lymphoproliferative neoplasms in chickens are often of viral origin, resulting from infection with Marek's disease virus, avian leukosis virus (ALV), or avian reticuloendotheliosis virus (REV) (Cadmus et al. 2019). In the poultry industry, the vaccination against Marek's disease and the eradication of ALV and REV viruses of the industrial breeds, may account for the few cases reported here. In contrast, for backyard flocks, vaccination is limited or poorly implemented, and the health status is unknown, potentially justifying the higher occurrence. A recent study on Marek's disease, conducted in our geographical region, demonstrated the presence of virulent viral strains in backyard chickens (Torres et al. 2019), as well as in another state (Chacón et al. 2019). Thus, it is essential to instruct also the backyard chicken owners about the importance of vaccination (Cadmus et al. 2019). In the present study, the differential diagnosis between avian leukosis and Marek's disease was based on histopathology and in the involvement of other tissues, beyond the liver. For Marek´s disease, lymphoid cells are markedly pleomorphic, with frequent involvement of peripheral nerves. In avian leukosis, there is proliferation of monomorphic round cells morphologically similar to lymphoblasts (lymphoma), without the involvement of nerves. The single diagnosis of primary liver neoplasm (hepatocellular adenoma) in a chicken of the current study, support the few cases reported (Williams et al. 2020).

Regarding infectious hepatic diseases, *Salmonella* spp. was the main bacterial agent diagnosed in the industrial layers and the backyard chickens. Based on the lesions found, together with epidemiological and laboratory data, all cases were associated with systemic salmonellosis. In addition, for cases of *Salmonella* Gallinarum biovar Gallinarum, chickens ages ranged from 8 to 77 weeks-old, in agreement with Shivaprasad (2000), in associating this biovar mainly with adult chickens. In these chickens, hepatomegaly was a frequent finding, as

well as hepatic color change, ranging from marked dark-red or dark-green to coppery. Some macroscopic findings are nonspecific and are also described in cases of *Escherichia coli* infection, such as hepatomegaly with multiple millimeter-sized foci of necrosis (Barcelos et al. 2006, Silva et al. 2012, Casagrande et al. 2017). However, the association of liver lesions with lesions in other organs may be indicative of infection by *Salmonella* spp. In the chickens of the present study, in addition to liver lesions, other changes such as splenitis, myocarditis (Shivaprasad 2000, Freitas Neto et al. 2007) and oophoritis (Dutta et al. 2015) were observed. The main histopathological findings were fibrinonecrotic and histiocytic hepatitis, and splenitis with vascular fibrinoid necrosis. Coagulation necrosis is commonly described in these cases, in addition to fibrinoid necrosis in the vascular wall (Shivaprasad 2000, Garcia et al. 2013).

Streptococcus spp. infection in the liver was diagnosed in 11 chickens in this study, seven broilers and four laying hens. In the present study, hepatomegaly was a frequent finding, and microscopically, the foci of necrosis were associated with intralesional bacteria (cocci) in most cases, in agreement with previously described findings (Chadfield et al. 2007, Borst 2020). Acute cholangio-hepatitis associated with gram positive cocci was diagnosed in two industrial layers. Similar lesions were previously associated with different bacteria such as *Escherichia coli*, *Staphylococcus* spp. (Barcelos et al. 2006) and *Clostridium perfringens* (Løvland & Kaldhusdal 1999).

Colibacillosis was diagnosed in mostly young broilers, industrial layers and backyard chickens of this study. In some of the broiler chicks, in addition to liver lesions, there was inflammation of the yolk sac, which may indicate the spread of the *E. coli* to the liver and other organs (Nolan et al. 2020). In these cases, yolk sac infection possibly occurred *in ovo*, as a consequence of salpingitis in the hens or at hatchery (eggshell contamination) (Vandekerchove et al. 2004). Enlarged liver and fibrinous peri-hepatitis (Nolan et al. 2020) were the main macroscopic findings. On histopathology, lesions found in the chickens of the current study were mainly fibrinous peri-hepatitis, peri-cholangitis and necrotic hepatitis, in agreement with previously described for colibacillosis (Barcelos et al. 2006, Casagrande et al. 2017). Other lesions found, such as splenitis, pericarditis and airsaccullitis, were also described in another study (Casagrande et al. 2017).

Viral hepatitis caused by an avian adenovirus in our study was found in 21-day-old broiler breeders. Necrotic hepatitis associated with basophilic and sometimes eosinophilic intranuclear corpuscles are findings consistent with IBH caused by avian adenovirus (Nakamura et al. 2011, Abdul-Aziz & Fletcher 2016). The disease has also been reported in young broilers (Fitzgerald 2020). The infection of broiler breeders may result in a vertical transmission (Fitzgerald 2020), highlighting the importance of monitoring adenovirus infection in breeders. Outbreaks of the disease have been documented in several countries, including Brazil. In the geographical region of this study, the FadV-D genome was detected in liver samples from layers, broilers, broiler breeders and backyard chickens (Pereira et al. 2014).

Regarding protozoan hepatitis, as by *Histomonas meleagridis* in backyard chickens, the infection was the least frequent cause for hepatitis. Most reports of this disease often occur in backyard chickens (Araújo et al. 2015, Brochu et al. 2019, Cadmus et al. 2019). However, there are also reports involving commercial poultry, such as industrial meat and breeding turkeys (Hauck et al. 2018), as well as layers from alternative farms (Stokholm et al. 2010). Outbreaks of histomoniasis can be related to alternative methods of rearing, in addition to the restricted use of medications. In the present study, the chickens lived along with ducks, geese, and guinea fowl, which are also susceptible to *Histomonas meleagridis* infection (Chute & Lund 1972, Lund et al. 1974) and to the cecal infection with the usually subclinical vector nematode *Heterakis gallinarum* (Schwarz et al. 2011). Necrotic typhlitis was found in two chickens in the present study, which has been described generally occurring concomitant with hepatitis (Hauck et al. 2018). However, in the chickens of our study, no infection was detected by *Heterakis gallinarum*, which is considered frequent in backyard chickens (Siqueira & Marques 2016) and closely associated with histomoniasis (Sentíes-Cué et al. 2009). Nevertheless, the cohabitation of chickens with other avian species may have been an important epidemiological factor for infection, as other poultry can be carriers of both infections (Clarke et al. 2017).

The limitations of the present study may be related to the representativeness of the sampled organs received for histopathology, considering the lack of important organs for examination, decreasing the accuracy and completeness of the histopathological description. In addition, few samples were sufficiently fresh or correctly collected in the field to enable the bacteriological examination.

CONCLUSIONS

Non-infectious causes were the most frequent in liver changes in this study, especially degenerative changes in broilers, and metabolic disease in layers and backyard chickens. Amyloidosis represents a metabolic disease currently manifestating in the form of outbreaks in laying hens, with evidence of economic loss due to abnormally increased mortality. Questions about the triggering factor causing hyperstimulation of the immune system, such as the type of vaccine, and the permanence of amyloid deposits in the liver, remain to be investigated.

Of the infectious causes, bacterial diseases, including salmonellosis, were the most frequent in our study. Some of the bacteria that cause hepatitis in poultry are relevant for poultry as well as in public health. Ancillary tests, such as bacteriological tests, are essential to confirm the etiology in bacterial hepatitis, since similar histopathological findings are common.

Finally, the results of this study provide parameters on the major hepatic diseases that affect broilers, laying hens, and backyard chickens, providing evidence to reinforce the prevention measures against infectious and non-infectious diseases.

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