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Characterization of coccidiosis and evaluation of suggestive cases of subclinical necrotic enteritis in broilers[1]

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ABSTRACT.- Santiani F., Silva R.O.S., Oliveira Júnior C.A., Withoeft J.A., Cristo T.G., Costa L.S., Gaspar T. & Casagrande R.A. 2023. **Characterization of coccidiosis and evaluation of suggestive cases of subclinical necrotic enteritis in broilers.** *Pesquisa Veterinária Brasileira 43:e07090, 2023.* Laboratório de Patologia Animal, Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina, Av. Luís de Camões 2090, Conta Dinheiro, Lages, SC 88520-000, Brazil. E-mail: renata.casagrande@udesc.br

This study performed the characterization of coccidiosis in broilers and evaluated the occurrence of suggestive cases of necrotic enteritis (NE), seeking if there is an association between the diseases in Brazilian flocks. Two hundred and fifty-six birds from 32 flocks were evaluated. Macroscopic and histopathological lesions were graduated for coccidiosis and NE. Intestinal content was investigated by polymerase chain reaction (PCR) for seven species of *Eimeria* and by selective anaerobic culture for *Clostridium perfringens* and identification of the NetB gene. Flocks positive for coccidiosis represented 93.8%. Macroscopic lesions of coccidiosis were Grade 1 for *E. acervulina* (27%); *E. tenella* (9.7%) and *E. maxima* (8.9%). Histopathological evaluation showed Grade 1 in duodenum (38.2%); jejunum (21.4%); cecum (9.3%) and ileum (5%). PCR demonstrated positivity for E. tenella (21.9%), *E. maxima* (18.8%), and *E. acervulina* (3.1%). Suggestive macroscopic lesions of necrotic enteritis ranged from Grade 1 (16%), 2 (23%) and 3 (10,9%). Histopathology indicated the absence of necrosis, showing only hemorrhage in the mucosa and submucosa, with the presence of *Eimeria* spp. *Clostridium perfringens* type A *netB*$^+$ was not isolated, demonstrating that macroscopic lesions found mostly in the jejunum did not characterize NE, based on histopathology and negativity of the NetB gene. The study suggests that, due to the high occurrence of coccidiosis, many macroscopic findings suggestive of NE are, in fact, attributed to atypical lesions caused by the reproduction of *Eimeria* spp.

INDEX TERMS: Coccidiosis, necrotic enteritis, broilers, eimeriosis, clostridiosis, genotyping, intestinal disease.

RESUMO.- [**Caracterização de coccidiose e avaliação de casos sugestivos de enterite necrótica subclínica em frangos de corte.**] Este estudo realizou a caracterização de coccidiose em frangos de corte e avaliou a ocorrência de casos sugestivos de enterite necrótica (EN), buscando se há alguma associação entre estas duas enfermidades em lotes de frango de corte no Brasil. Foram avaliadas 256 aves de 32 lotes. Lesões macroscópicas e histopatológicas foram graduadas para coccidiose e EN. O conteúdo intestinal foi investigado por reação em cadeia da polimerase (PCR) para sete espécies de *Eimeria* e por cultura anaeróbia seletiva para *Clostridium perfringens* e identificação do gene NetB. Os lotes positivos para coccidiose representaram 93,8%. Lesões macroscópicas de coccidiose foram de Grau 1 para *E. acervulina* (27%); *E. tenella* (9,7%) e *E. maxima* (8,9%). A avaliação histopatológica mostrou Grau 1 no duodeno (38,2%); jejuno (21,4%); ceco (9,3%) e íleo (5%). A PCR demonstrou positividade para *E. tenella* (21,9%), *E. maxima* (18,8%) e *E. acervulina* (3,1%). Lesões macroscópicas sugestivas de enterite necrótica variaram de grau 1 (16%), 2 (23%) e 3 (10,9%).

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A histopatologia indicou ausência de necrose, apresentando apenas hemorragia em mucosa e submucosa, com presença de *Eimeria* spp. *Clostridium perfringens* tipo A netB + não foi isolado, demonstrando que lesões macroscópicas encontradas principalmente no jejuno não caracterizaram NE, com base na histopatologia e negatividade do gene NetB. O estudo sugere que, em virtude da alta ocorrência de coccidiose nos lotes, muitos achados macroscópicos sugestivos de EN são, na verdade, atribuídos a lesões atípicas provocadas pela reprodução de *Eimeria* spp.

TERMOS DE INDEXAÇÃO: Coccidiose, enterite necrótica, frangos de corte, eimeriose, clostridiose, genotipagem, doença intestinal.

INTRODUCTION

Coccidiosis is an enteric disease caused by protozoa of the *Eimeria* genus. The species that affect broiler chicken are *E. acervulina*, *E. maxima*, *E. tenella*, *E. brunetti*, *E. praecox*, *E. necatrix*, and *E. mitis*, with location and macroscopic patterns that often indicate the species involved. The first three species cause typical lesions in the duodenum, jejunum, and cecum, respectively, and have the highest occurrence in broiler flocks, commonly monitored (Chapman 2014). Due to the damage caused to the intestinal epithelium by coccids and changes in gastrointestinal functions, like an exacerbated response to mucus production when enterocytes are attacked by coccids. It can predispose to secondary diseases, including infections caused by *Clostridium perfringens*, which has a strong acidomucolytic activity, using this alteration for its installation (Collier et al. 2008). *Clostridium perfringens* is a commensal inhabitant of the gastrointestinal tract of birds but is also responsible for causing necrotic enteritis (NE) with intestinal lesions in duodenum, jejunum, ileum, and cecum (Cooper et al. 2013). Acute lesions include friability of the small intestine, filled with reddish or dark brown content, and yellowish diphtheria pseudomembranes forming over the mucosal associated with multifocal to coalescent ulcers (Cooper et al. 2013), discretely present also in its subclinical form (Gholamiandehkordi et al. 2007).

The diagnosis of NE must be based on several criteria. Macroscopic and histopathological lesions are necessary to rule out other diseases that cause similar lesions, such as coccidiosis (McDougald 2003, Collier et al. 2008). Once *C. perfringens* type A is part of the intestinal microbiota, its isolation is not enough to complete the diagnosis. Thus, the detection of the NetB encoding gene, a pore-forming toxin associated with the development of NE, in *C. perfringens* isolates, using the polymerase chain reaction (PCR), is the best approach to diagnosis (Keyburn et al. 2008). Thus, the objective of this work was to carry out the macroscopic and histological characterization of cases of coccidiosis and if macroscopically suggestive cases of necrotic enteritis really are NE through molecular analysis, evaluating if there is an association between these intestinal disorders in broilers in Brazil.

MATERIALS AND METHODS

Sampling. The experiment consisted of 32 flocks of female Cobb 500™ broilers, with an average age of 29 days, obtained from a slaughterhouse in Santa Catarina State, Southern Brazil. The samples were a set of viscera from each broiler, obtained from the slaughter line. Eight birds were evaluated for each flock, four without visible macroscopic changes in the intestines, and four presenting lesions in the intestine, totaling 256 birds.

Macroscopic and histological characterization of the intestinal lesions. The macroscopic analysis of the intestines for NE was classified according to the lesion score based on adapted classification method (Truscott & Al-Sheikhly 1977), with Grade 0: no lesions; Grade 1: circular, depressed, and reddish areas with 1 to 2mm in diameter, transmural, which may be covered by fibrillar, irregular, and yellowish material, mainly in the jejunum and ileum; Grade 2: circular, depressed, reddish to blackish areas and ulcerations with a hyperemic halo, from 2 to 5mm in diameter, transmural, covered by fibrillar, irregular, and yellowish material, mainly in jejunum and ileum; Grade 3: areas greater than 5mm, red or purplish, transmural, with deposition of fibrillar, irregular, and yellowish material, mainly in the jejunum and ileum, predominantly on Peyer plates. Duodenum, jejunum, ileum, cecum, and colon fragments measuring approximately 3cm were fixed in 10% buffered formalin, routinely processed for histopathology, and stained with hematoxylin and eosin (HE).

The intestines were evaluated for investigation of lesions characteristic of coccidiosis, graded using an adapted methodology (Johnson & Reid 1970). Microscopic changes caused by coccidiosis in intestines received a lesion score consisting of Grade 0: no changes; Grade 1: slight amount of parasitic structures (schizonts, micro and macrogametes, and oocysts) compatible with *Eimeria* spp. in the top superficial layer and medial region in less than ten villi or crypts; Grade 2: a moderate to a large number of parasitic structures compatible with *Eimeria* spp. in less than ten villi or crypts; Grade 3: a moderate to a large number of parasitic structures compatible with *Eimeria* spp. in the superficial layer in more than ten villi or crypts; and Grade 4: 80% or more of the villi or crypts affected by parasitic structures compatible with *Eimeria* spp. The remaining changes observed in the intestines that contained lesions suggestive of NE were assigned Grade 0 for the absence of histological changes; Grades 1 to 3 representing, respectively, mild to moderate multifocal hemorrhage, accentuated multifocal hemorrhage, and diffuse moderate to severe hemorrhage.

Parasitological examination for *Eimeria* spp. The search for oocysts of *Eimeria* spp. was performed by collecting a pool of intestinal contents during macroscopic evaluation and the number of excreta was variable depending on how full the bird's intestine was during necropsy, with an adapted methodology from Moraes et al. (2015), which consisted of a stage in which the oocysts were purified and cleaned, followed by DNA extraction with phenol-chloroform procedure and multiplex PCR for the seven species of *Eimeria*, with the primers ac-A03-F and ac-A03-R (5'-AGTCAGCCACACAATAATGGCAAACATG-3' and 5'-AGTCAGCCACAGCGAAAGACGTATGTG-3') for *E. acervulina*, resulting in an amplicon with 811bp; br-J18-F and br-J18-R (5'-TGGTCGCAGAACCTACAGGGCTGT-3' and 5'-TGGTCGCAGACGTATATTAGGGGTCTG-3') for *E. brunetti*, resulting in an amplicon with 626bp; tn-K04-F and tn-K04-R (5'-CCGCCCAAACCAGGTGTCACG-3' and 5'-CCGCCCAAACATGCAAGATGGC-3') for *E. tenella*, resulting in an amplicon with 539bp; mt-A03-F and mt-A03-R (5'-AGTCAGCCACCAGTAGAGCCAATATTT-3' and 5'-AGTCAGCCACAAACAAATTCAAACTCTAC-3') for *E. mitis*, resulting in an amplicon with 460bp; pr-A03-F and pr-A03-R (5'-AGTCAGCCACCACCAAATAGAACCTTGG-3' and 5'-GCCTGCTTACTACAAACTTGCAAGCCCT-3') for *E. praecox*, resulting in an amplicon with 354bp; mx-A09-F

and mx-A09-R (5'-GGGTAACGCCAACTGCCGGGTATG-3' and 5'-AGCAAACCGTAAAGGCCGAAGTCCTAGA-3') for *E. maxima*, resulting in an amplicon with 272bp; nc-A18-F and nc-ENec-R (5'-TTCATTTCGCTTAACAATATTTGGCCTCA-3' and 5'-ACAACGCCTCATAACCCCAAGAAATTTTG-3') for *E. necatrix*, resulting in an amplicon with 200bp. The reaction was performed in a thermal cycler programmed for the conditions of 96°C for 5 min, 35 cycles of 1 min at 94°C and 2 min at 65°C, and a final extension of 10 min at 72°C. The products were subjected to electrophoresis in 2% agarose gel for visualization.

Isolation and genotyping of *C. perfringens*. A fragment of intestine measuring 2-3cm, sampled from each bird that presented macroscopic lesions, was connected using cotton string, the ends of the loop were sectioned, and the sample was individually packed in plastic bags. A fragment of the jejunum was also collected from the four birds that did not show intestinal lesions. All intestine fragments were frozen at -20°C for further microbiological analysis. The samples were processed at the Anaerobic Laboratory of the "Universidade Federal de Minas Gerais" (UFMG). Each sample was subjected to enrichment and selective cultivation as previously described (Silva et al. 2016). Briefly, an aliquot of intestinal content was inoculated into Brain and Heart Infusion broth (OXOID®) and incubated in an anaerobic chamber (Forma Anaerobic System, Thermo Scientific®) at 37°C for 24 hours. Thus, each sample was plated on Shahidi Ferguson Perfringens agar (DIFCO™) and incubated again in an anaerobic chamber at 37°C for 48 hours. Sulfite-reducing colonies were submitted to a multiplex PCR for genotyping (Vieira et al. 2006) and to a monoplex PCR for detection of *netB* (Keyburn et al. 2008), using the primers AKP78 and AKP79 (5′-GCTGGTGCTGGAATAAATGC-3′and 5′-TCGCCATTGAGTAGTTTCCC-3′, respectively).

Statistical analysis. The data were analyzed using spreadsheets made in *Excel* and descriptive and inferential statistics. Statistical analysis was performed to compare the degrees of macroscopic and histopathological lesions using the Wilcoxon test at a significance level of 0.05, using Sigma Plot software, version 12.0.

Bioethics and Biosecurity Committee Approval.- This study was approved by the Ethics Committee on the Use of Animals (CEUA) of the "Universidade do Estado de Santa Catarina" (UDESC) under number 9977130319.

RESULTS

Evaluation of the macroscopic and histopathological lesions. Among the evaluated flocks, 93.8% (30/32) were positive for coccidiosis (Table 1), with macroscopic and histopathological lesions shown in Figure 1-6. Of the 256 intestine samples evaluated, 50% (128/256) presented no NE suggestive macroscopic lesions (Grade 0). Grade 1 was found in 16% (41/256), Grade 2 in 23% (59/256) and Grade 3 in 10.9% (28/256) (Table 2). The main changes found in this evaluation were hemorrhages in the mucosa and submucosa sometimes accompanied by parasitic structures compatible with *Eimeria* spp., without evidence of necrosis (Figure 7-12). In the jejunum sections that received Grade 0 for NE during the histopathological evaluation, parasitic structures of *E. maxima* were observed in 14.4% (37/256), while 7.8% (20/256) received Grade 1, 3.5% (9/256) Grade 2, and 3.1% (8/256) Grade 3.

***Eimeria* spp. oocysts analysis by PCR.** *Eimeria* oocysts were identified in 34.3% (11/32) of the flocks, of which 21.9% (7/32) were *E. tenella*, 18.8% (6/32) were *E. maxima*, and 3.1% (1/32) were *E. acervulina*. Mixed infection was observed in three flocks, two of which consisted of *E. maxima* and *E. tenella*, and one of *E. acervulina* and *E. tenella*.

Isolation and genotyping of *Clostridium perfringens*. Of the intestine samples submitted to selective growth in an anaerobic environment, 8.2% (21/256) were positive for *C. perfringens* type A, all of which were negative for the NetB gene. Among these 21 positive samples, 11 had macroscopic lesions suggestive of NE, with five samples considered Grade 1, five as Grade 2, and one as Grade 3. The histopathological evaluation showed mucosa and submucosal hemorrhage in 10 samples, three of which were accompanied by *Eimeria* spp. parasitic structures, while one sample showed no histological changes. The remaining ten samples showed Grade 0. However, the histopathological examination showed that four samples contained parasitic structures compatible with *Eimeria* spp.

DISCUSSION

Eimeria acervulina, E. maxima, and *E. tenella* were the species most found in this study. They are the species most found in broilers naturally infected, through the macroscopic lesion

Table 1. Evaluation of coccidiosis in broilers obtained from a slaughterhouse in Brazil. Scores of macroscopic and histopathological lesions (n = 256 samples)

Grade	Macroscopic lesions					Microscopic lesions				
	Duodenum	Jejunum	Ileum	Cecum	Colon	Duodenum	Jejunum	Ileum	Cecum	Colon
G0	64% (164)a	90.6% (232)a	100% (256)a	90.2% (231)a	99.2% (254)a	45.7% (117)b	62.5% (160)b	92.9% (238)b	83.9% (215)a	99.7% (255)a
G1	27% (70)a	8.9% (23)a	0a	9.7% (25)a	0.7% (2)a	38.2% (98)b	21.4% (55)b	5% (13)b	9.3% (24)a	0.3% (1)a
G2	7% (18)a	0.3% (1)a	0a	0a	0	8.9% (23)a	5.4% (14)b	0.7% (2)a	1.5% (4)a	0
G3	1.5% (4)a	0a	0a	0a	0	7% (18)b	10.5% (27)b	1.1% (3)a	5% (13)a	0

Different letters in the same line and corresponding column indicate a significant difference (p<0.05); Duodenum = Eimeria *acervulina,* Eimeria *mitis and* Eimeria *praecox,* Jejunum = Eimeria *maxima,* Ileum = Eimeria *necatrix,* Cecum = Eimeria *tenella,* Colon = Eimeria *brunetti*.

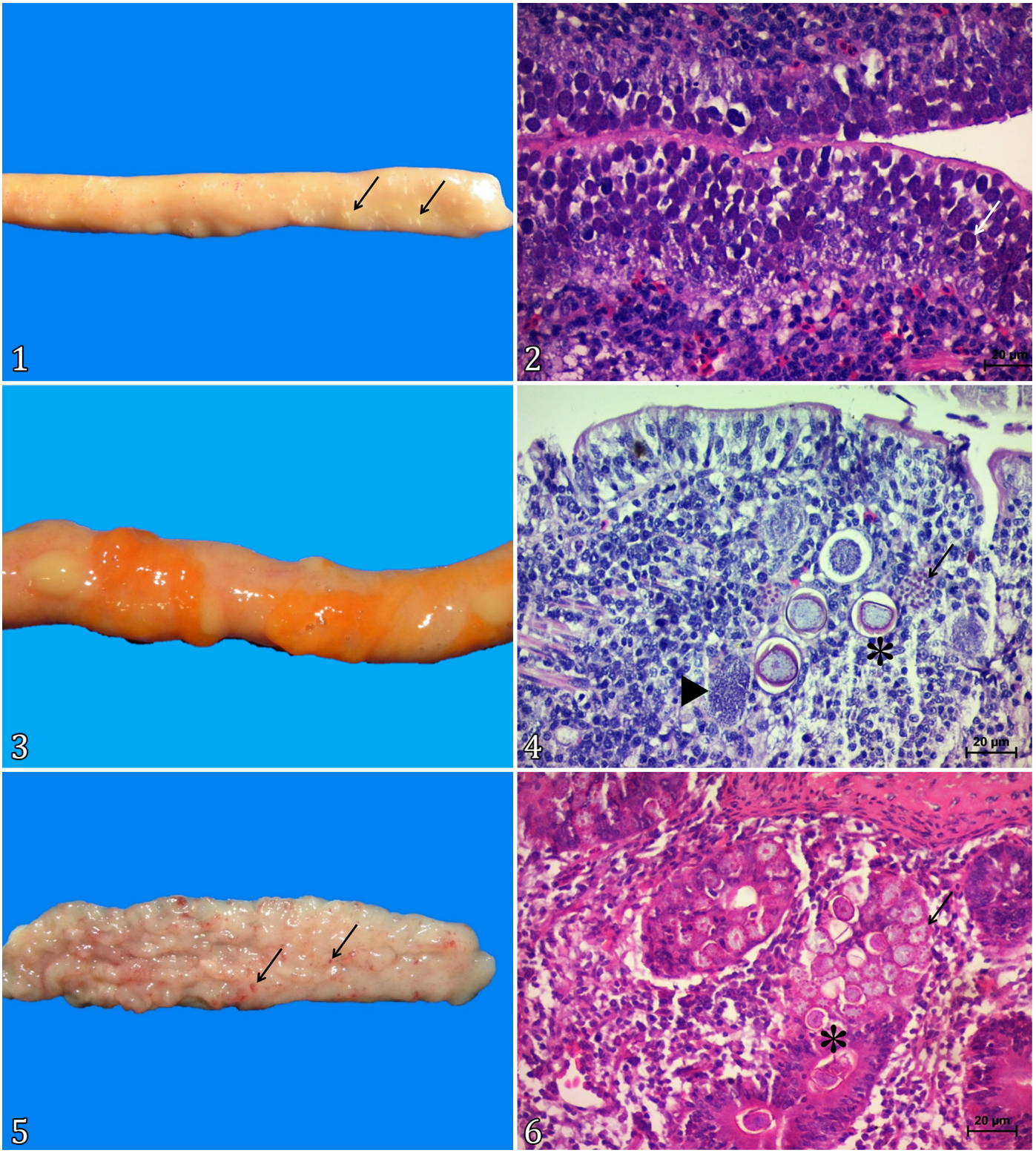

Fig.1-6. Macroscopic and histopathological lesions of coccidiosis in broilers. (**1**) Duodenum: discrete multifocal whitish transverse streaks (arrows) (*Eimeria acervulina* Grade 1). (**2**) Duodenum: the slight amount of parasitic structures comprising schizonts (arrow) in the mucosa at the top of the villi (Grade 1). HE, obj.40x. (**3**) Jejunum: orange mucous intestinal content in the mucosa, slight multifocal (*Eimeria maxima* Grade 1). (**4**) Jejunum: the slight amount of parasitic structures containing schizonts (arrowhead), macrogametocytes (arrow), and immature oocysts (asterisk) in the submucosa (Grade 1). HE, obj.40x. (**5**) Cecum: slight multifocal red areas (arrows) in the mucosa (*Eimeria tenella* Grade 1). (**6**) Cecum: the slight amount of parasitic structures containing macrogametocytes (arrow) and immature oocysts (asterisk) in the mucosa and submucosa (Grade 1). HE, obj.40x.

score (Carvalho et al. 2011). In Southern Brazil, a prevalence of 63.3% was found for *E. acervulina* (Moraes et al. 2015) and 30% in North East (Carvalho et al. 2011). *E. maxima* and *E. tenella* demonstrates a similar trend (Carvalho et al. 2011, Moraes et al. 2015). The microscopy score showed a greater number of birds with coccidiosis lesions in the duodenum, jejunum, and ileum compared to the macroscopic score. It is known that histopathology, which is widely used for academic or research purposes, has greater sensitivity and allows differentiating the stages of the cycle of *Eimeria* sp. (Kawazoe 2009).

Previous studies have suggested that the evaluation of macroscopic lesions alone is not accurate for the diagnosis of coccidiosis, once it is rarely able to demonstrate the severity and extent of the lesions (Conway et al. 1999). Studies using histopathological evaluation for the diagnosis of coccidiosis are scarce and, when used, do not use a standardized classification (Kawazoe 2009). In all evaluations, it was found that Grade 1 was the most prevalent, showing a mild form of the disease. The PCR results for the identification of *Eimeria* obtained in the present study were lower when compared to the results obtained in other studies with the same objective (Haug et al. 2008, Moraes et al. 2015). The number of oocysts in the samples is variable, which can directly interfere with the amount of DNA present, compromising the sensitivity of the PCR since amplification requires at least 3.2 sporocysts per *Eimeria* species (Haug et al. 2008). The samples used in this study were obtained from the intestinal contents of eight broilers, however, these birds were subjected to pre-slaughter fasting, which may also have interfered with the number of oocysts present. A lower number of cases with *E. acervulina* detection by PCR was observed in relation to histopathology, which can be explained by samples with few oocysts, residues remaining from oocyst purification, or by the presence of PCR inhibitors in manure samples of birds and may interfere with DNA extraction (Jenkins et al. 2006, Haug et al. 2008).

Coccidiosis is also the most important disease as a differential diagnosis of subclinical NE, which resembles the macroscopic lesions found in the present study. However, the primary changes indicated by the histopathological evaluation consisted of hemorrhages and parasitic structures compatible with *Eimeria* spp. NE is typically characterized by mucosal necrosis, usually restricted to the villi, presenting coagulation necrosis and desquamation of epithelial cells. As it progresses, the infiltration of inflammatory cells occurs, delimiting the necrotic area, with heterophile and macrophages being the predominant, and cell debris, bacilli, and fibrin deposition can be found in the lumen (Cooper et al. 2013). In this report, the histopathological evaluation showed no lesions compatible with NE.

In this study, *Clostridium perfringens* type A was isolated, which is known as the predominant genotype in poultry. However, as it is a commensal bacteria of the broilers microbiota, its isolation alone is not enough to diagnose NE. The alpha-toxin, produced by all *C. perfringens* types was considered for many years as the main virulence factor of NE in poultry, but its importance was questioned since *netB*, a novel pore-forming toxin associated with NE was described (Keyburn et al. 2008, Cooper et al. 2013). Since then, many studies showed that *C. perfringens* isolates from chickens suffering from NE are commonly *netB*+. Furthermore, experimentally, *netB*+ strains can cause NE even in absence of alpha-toxin production, providing enough evidence that *netB* is an essential virulence factor in the pathogenesis of NE (Keyburn et al. 2010). In layer pullets, a study showed that cases of NE were detected without the *netB* gene, however, with histopathological lesions compatible with NE, with *Clostridium*-like bacterial structures deposited in the necrotic tissue, suggesting that, in laying pullets, the disease can occur without the manifestation of the toxin (Goossens et al. 2020). In the present study, *netB* was not detected in any of the C*. perfringens* type A isolates, agreeing with the absence of histological lesions, which excluded the NE from the differential for macroscopic lesions. There are still no reports on the presence of *C. perfringens netB*+ isolates in broilers in Brazil, demonstrating that, as in this study, many cases macroscopically suggestive of NE may actually be related to other diseases, like coccidiosis.

Many studies consider the crucial role of *Eimeria* spp. in the development of NE, in an isolated or multifactorial form (Collier et al. 2008, Cooper et al. 2013), and its known that layer flocks diagnosed with eimeriosis are two-fold more likely to develop NE (Goossens et al. 2020). The positivity of 30/32 flocks for coccidiosis could be a triggering factor

Table 2. Occurrence of coccidiosis and suggestive cases of necrotic enteritis in Brazil: macroscopically suggestive cases of necrotic enteritis and their histopatological findings

	Macroscopic lesions			Histopathological lesions					
				Haemorrhage			*Eimeria* spp.		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
Duodenum	3.9% (10)*	0	0.4% (1)	1.6% (4)	0	0.4% (1)	0.4% (1)	0	0.8% (2)
Jejunum	8.2% (21)	16.8% (43)	9.8% (25)	23.4% (60)	0.4% (1)	5.8% (15)	7.4% (19)	0	3.5% (9)
Ileum	3.9% (10)	6.2% (16)	0.8% (2)	6.2% (16)	0.8% (2)	0.4% (1)	0.8% (2)	0.4% (1)	0
TOTAL	16% (41)	23% (59)	10.9% (28)	32% (80)	1.2% (3)	6.6% (17)	8.6% (22)	0.4% (1)	4.3% (11)

* Numbers in parentheses indicate the absolute frequency of the cases.

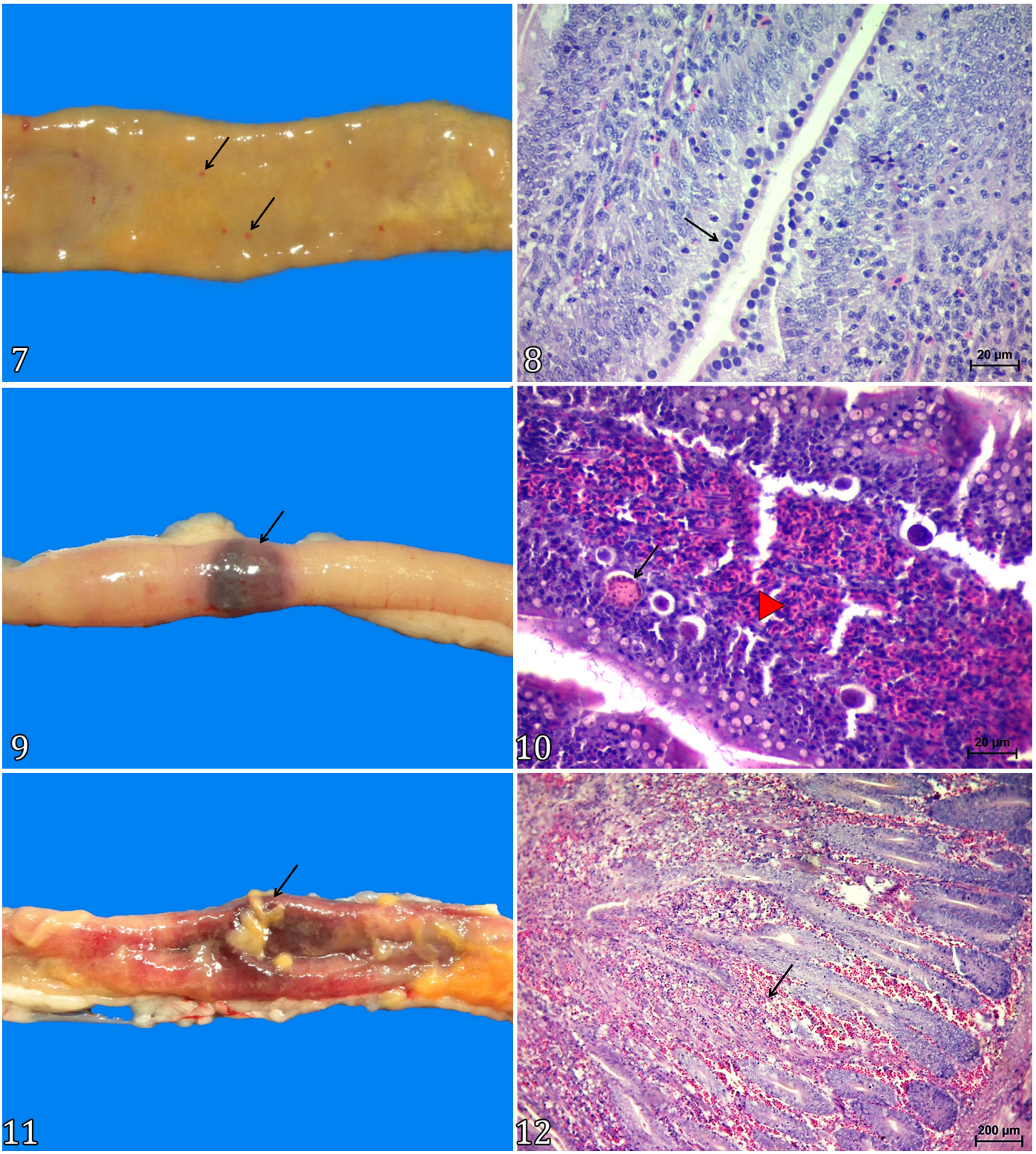

Fig.7-12. Macroscopic lesions suggestive of necrotic enteritis (NE) and intestinal histopathological lesions in broilers. (**7**) Duodenum: reddish areas in the mucosa, up to 2mm in diameter (arrows), slight multifocal (Grade 1). (**8**) Duodenum: the slight amount of parasitic structures containing schizonts (arrow) at the top and superficial region of the villi, compatible with *Eimeria* spp. Grade 1. HE, obj.40x. (**9**) Ileum: reddish area in the serosa, measuring 5mm in diameter (arrow), moderate focal (Grade 2). (**10**) Ileum: the slight amount of macrogametocytes (arrow) and immature oocysts in the medial region of the villi, compatible with *Eimeria* spp. Grade 1 and moderate focal hemorrhage (arrowhead) Grade 2. HE, obj.40x. (**11**) Jejunum: reddish area with deposition of fibrillar material on the mucosal surface (arrow), sharp focal (Grade 3). (**12**) Jejunum: accentuated diffuse hemorrhage (arrow) Grade 3. HE, obj.10x.

for NE. However, the histopathological evaluation and the absence of *C. perfringens netB*+ excluded NE as the cause of the intestinal lesions. The lesions were found mainly in the jejunum, where it is possible to observe hemorrhagic lesions due to infection by *E. maxima* and *E. necatrix*. In this intestinal segment, the greatest tissue lesions promoted by *E. maxima* occur during sexual reproduction, when the formation of subepithelial macrogametocytes, close to blood vessels, can cause hemorrhages and fluid losses (McDougald 2003). Therefore, the lesions found in the small intestine can be derived from atypical macroscopic lesions of coccidiosis, which are not present in the macroscopic lesion score (Johnson & Reid 1970).

CONCLUSION

The occurrence of coccidiosis in the evaluated flocks was high, showing predominantly classified as Grade 1. The histopathological evaluation was superior to macroscopy and PCR for the diagnostic confirmation of coccidiosis in broilers. The histological lesions observed did not suggest the occurrence of NE in these flocks, which corroborates with the absence of *Clostridium perfringens* type A *netB*+ in the samples. Due to the high positivity for coccidiosis, the lesions observed in the small intestine may be atypical lesions of the disease. The association of different methods of diagnosis of NE is important since only macroscopic lesions can be flawed for its diagnosis.

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