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Pathological changes in palatine tonsils of slaughtered water buffaloes (*Bubalus bubalis*)[1]

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ABSTRACT.- Teixeira M.A.S., Moura M.A.O., González G.B.E., Pires C.R.S., Santos T.F.S., Riet-Correa G., Cerqueira V.D. & Bezerra Júnior P.S. 2024. **Pathological changes in palatine tonsils of slaughtered water buffaloes (*Bubalus bubalis*).** *Pesquisa Veterinária Brasileira 44:e07285, 2024.* Graduate Program in Animal Health in the Amazon, Laboratório de Patologia Animal, Instituto de Medicina Veterinária, Universidade Federal do Pará, Campus Castanhal, BR-316 Km 61, Bairro Saudade, Castanhal, PA 68746-360, Brazil. E-mail: pedrobezerra@ufpa.br

This study evaluated palatine tonsils from 327 water buffaloes collected in a slaughterhouse located in the city of Belém, Pará State, Brazil, over four months (nine sample collecting visits). The overwhelming majority (84.71%) of these buffaloes came from Marajó Island, Pará State, Brazil. The main pathological findings were crypt dilatations/keratin cysts (96.0%, 314 cases), mild to moderate follicular lymphoid hyperplasia (93.0%, 304 cases), crypt abscesses (79.8%, 261 cases), tonsillar corpuscles (31.2%, 102 cases) and tuberculoid granulomas (1.53%, 5 cases). Acid-fast bacilli (AFB) were observed in 20% (1 out of 5 cases) of these granulomas. The study indicates that pathological evaluation of tonsils in buffaloes can provide details of structural features of the organ in the species and information on subclinical or asymptomatic tonsillitis.

INDEX TERMS: Buffaloes, *Bubalus bubalis,* tonsils, lesions, tuberculosis, abscesses.

RESUMO.- [**Alterações patológicas em tonsilas palatinas de bubalinos (*Bubalus bubalis*).**] No presente estudo foram avaliadas tonsilas palatinas de 327 bubalinos, coletadas em nove visitas a um abatedouro frigorífico localizado na cidade de Belém, no estado do Pará, Brasil, em um período de quatro meses. Estes bubalinos, em sua ampla maioria (84,71%), eram procedentes da Ilha de Marajó, no estado do Pará, Brasil. Os principais achados patológicos foram dilatações de criptas/cistos de queratina (96,0%, 314 casos), hiperplasia linfoide folicular leve a moderada (93,0%, 304 casos), abscessos de criptas (79,8%, 261 casos), corpúsculos tonsilares (31,2%, 102 casos) e granulomas tuberculoides (1,53%, 5 casos). Bacilos álcool-ácido resistentes (BAAR) foram observados em 20% (1 de 5 casos) destes granulomas. O estudo indica que a avaliação patológica das tonsilas de bubalinos pode fornecer detalhes de características estruturais do órgão na espécie e informações de tonsilites subclínicas ou assintomáticas.

TERMOS DE INDEXAÇÃO: Búfalos, *Bubalus bubalis,* amígdalas, lesões, tuberculose, abscessos.

INTRODUCTION

Buffaloes (*Bubalus bubalis*) are animals of Asian origin, also referred to as water buffalo or Asian buffalo. There are roughly one million four hundred thousand buffaloes in Brazil, 38.13% of which are found in the state of Pará, where they are used for meat and milk production, riding, and drafting (IBGE 2019).

Anatomically, the palatine tonsils are secondary lymphoid organs located at the intersection of the digestive and respiratory tracts, comprising the mucosa-associated lymphoid tissue (MALT). Their strategic location allows them to come into contact with ingested or inhaled agents. They feature tissue formed by branched tonsillar crypts bordered by lymphoid tissue. The crypts are covered with non-keratinized stratified squamous epithelium. In some regions, the epithelium has interstices through which lymphoid cells circulate and is

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referred to as lymphoepithelium or crypt-reticulated epithelium (Perry & Whyte 1998, Kumar et al. 2008, Zidan & Pabst 2011). In older buffaloes, this epithelium is thicker (Girgiri & Kumar 2018). Bubalines have relatively larger (Girgiri & Kumar 2021) and double (Zidan & Pabst 2011) crypts, with transverse folds of various shapes and sizes in the mucosa (Girgiri & Kumar 2021).

There are several studies on tonsillar lesions in the human species (Wilkinson 1929, Tan et al. 2018), but considerably fewer studies in animals. In bovines, the tonsils have been reported as sites of proliferation of several agents (Liebler-Tenorio et al 1997, Briggs et al. 1998, Zhang & Alexandersen 2004). In buffaloes, data concerning pathological findings in the palatine tonsils are scarce, but these are important in the pathogenesis of diseases such as tuberculosis and pasteurellosis (De Vos et al. 2001, De Klerk et al. 2006, Annas et al. 2014). The palatine tonsils of ruminants are among the specified risk materials (SRM) for bovine spongiform encephalopathy (BSE) and, therefore, there is an indication for their extirpation from the carcasses, allowing for morphological studies and the search for infectious agents in reasonable equilibrium with the organism. The objective of the present study was to research macroscopic and histological lesions in palatine tonsils of slaughtered buffaloes in the city of Belém, Pará State, Brazil.

MATERIALS AND METHODS

Animal Ethics. This study is in accordance with the precepts of Act No. 11,794, dated October 8, 2008, with Decree No. 6.899, dated July 15, 2009, and with the standards edited by the National Council for the Control of Animal Experimentation (CONCEA). The project was submitted and approved, under number 7570261120, by the Ethics Committee on Animal Use of the "Universidade Federal do Pará" (CEUA-UFPA).

Sample size and sampling. The average monthly number of buffaloes slaughtered at the facility over 2018 (n=1012) was considered the target population to calculate the sample size. The Statcalc tool of the EpiInfoTM7.2.2.16 software was used for the calculation, considering an expected frequency of 50%, an acceptable error of 5%, and a confidence interval of 95%. Thus, reliably, the minimum sample size should be 278 animals.

Collection and processing of biological material. The samples were collected in nine visits by convenience to the slaughterhouse from December 2, 2019, to April 1, 2020, and tonsils were collected from 327 adult buffaloes.

In the slaughterhouse, after being removed from the carcasses, the organs were placed in plastic bags and kept under refrigeration until they were received at the laboratory. Then, they were identified, sectioned lengthwise, and fixed in a 10% formaldehyde solution buffered with phosphates for 24 to 48 hours. After that, the fragments were routinely processed for histopathology, stained with hematoxylin and eosin (HE) and visualized under an optical microscope. Selected sections were submitted to Ziehl-Neelsen and Von Kossa staining.

Classification of lesions. The histopathological classification of the changes was based largely on a review study of human tonsil lesions (Tan et al. 2018). The lesions observed in the herein study were classified as to type, intensity, and distribution. As for the type, crypt abscesses, hyperplasia of lymphoid follicles, dilatation and keratin cysts in the crypts, granulomatous inflammation and tonsillar corpuscles were observed. The intensity of the lesions was qualitatively evaluated as mild, moderate, and/or severe. As for distribution, the lesions were classified as focal or multifocal. In crypt dilatations and cysts, the presence and amount of keratinocytes or keratin in the lumen and the shape of the dilated crypt were considered to determine the level of the lesion. In lesions considered to be mild dilatation of the crypts, there was a small number of nucleated keratinocytes in the lumen and crypts with edges forming acute angles. In the moderate dilatations of the crypts, there were large numbers of nucleated keratinocytes filling the crypts, which had rounded shapes forming obtuse angles. In the marked dilatations of crypts (keratin cysts), there was a large quantity of keratin with few nucleated cells in the lumen of the crypts, which had a rounded shape. The lesions were classified as crypt abscesses when there was an inflammatory infiltrate with a predominance of polymorphonuclear cells (neutrophils and/or eosinophils) in the lumen of the crypts. Tonsillar corpuscles were considered rare when there were fewer than five structures per slide, occasional when there were six to 10, and frequent when there were more than 10.

The macroscopic aspects of the lesions were obtained from the formalin-fixed specimens after histopathological classification. For the photographic recording of the macroscopic lesions, to obtain a stain closer to the fresh tissue, the fixed pieces were immersed in 70% ethyl alcohol for 24 to 48 hours.

RESULTS

For this study, the palatine tonsils of 327 male and female buffaloes of mixed breeds, aged between two and 10 years, were analyzed. Of this total, 277 (84.71%) were from the Marajó Archipelago (Fig.1).

The frequency, distribution, and intensity of lesions classified as tonsillar crypt dilatations (Fig.2-5) and abscesses (Fig.6-9) are shown in Table 1 and 2. The most frequent changes in the palatine tonsils in the present study were dilations of tonsillar crypts, observed in 96.02% (314/327) of the buffaloes. In 40.13% (126/314) of these, there was only mild dilation; in 37.9% (119/314), dilation was moderate; and in 21.97% (69/314), dilation was marked. Hyperplasia of lymphoid follicles was observed in 304 (92.97%) buffaloes. In 195 (64.14%) of these animals, the lesion was considered moderate, and in 109 (35.86%), it was considered mild.

In five (1.53%, 5/327) buffaloes in the study, there were granulomatous lesions in the tonsils compatible with tuberculosis. In four (1.22%, 4/327) of these buffaloes, macroscopic lesions compatible with tuberculosis were observed in other organs, with total condemnation of the carcasses. In only one (0.31%, 1/327) of these cases was the granulomatous lesion seen only in the tonsils. The granulomatous lesions observed were characterized by caseous necrosis in the palatine tonsil's parenchyma, with mineralization foci. On microscopy, these areas of necrosis were bordered by macrophages, epithelioid cells, Langhans giant cells and lymphocytes (Fig.10-13). Acid-fast bacilli (AFB) were found in 20% (1/5) of these palatine tonsils.

Tonsillar corpuscles were observed in 31.19% (102/327) of the buffaloes in the study. In 52 (50.98%) animals, the corpuscles were considered rare; in 31 (30.39%) occasional, and in 19 (18.33%), they were frequent. The tonsillar corpuscles were round and basophilic structures, occasionally with multiple layers concentric to a central nucleation, and calcium salts in these structures were confirmed by Von Kossa stain. These structures were observed in the basal layer of the crypt epithelium, in the underlying lymphoid tissue, and less frequently inside the crypts. Individual or small groups of keratinized epithelial cells were observed in the basal layer in two animals (Fig.14-17).

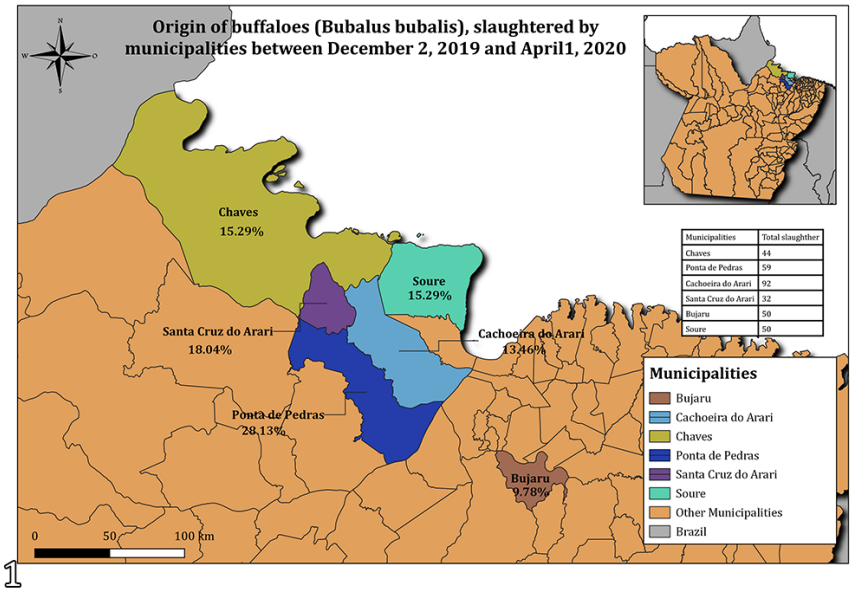

Fig.1. Pathological changes in palatine tonsils of buffaloes. Map of the origin of the buffalo included in the study, in Castanhal/PA, 2022.

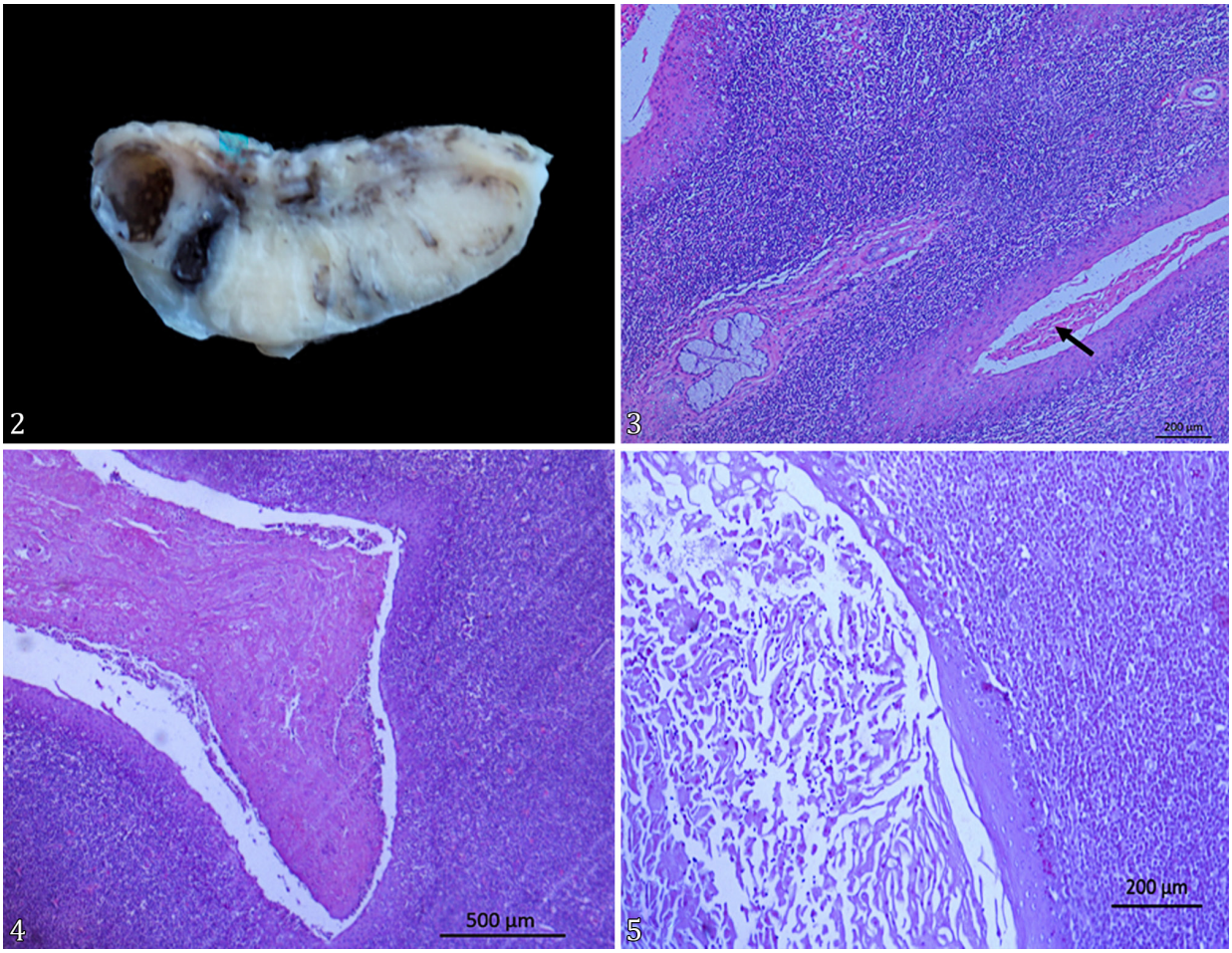

Fig.2-5. Pathological changes in palatine tonsils of buffaloes. Palatine tonsil of a buffalo. Keratin dilatation and cyst in the crypts. (**2**) Buffalo TO 211: Keratin cyst partially filled with brownish gelatinous material. (**3**) Buffalo TO 203: Slight dilatation of the crypt, with small numbers of nucleated keratinocytes in the lumen (arrow), with a margin below forming an acute angle. HE, obj.10x. (**4**) Buffalo TO 99: Moderate dilatation of the crypt, with large numbers of nucleated keratinocytes in the lumen, with a rounded crypt margin above and an obtuse angle below. HE, obj.5x. (**5**) Buffalo TO 54: Marked dilatation of the crypt (keratin cyst), lumen containing a large amount of keratin, with a small number of nucleated cells; note rounded edges of the crypt. HE, obj.10x.

DISCUSSION

The most frequent changes in buffaloes were dilations of tonsillar crypts (96.02%), similar to a study in humans by Tan et al. (2018), where simple cysts filled with keratin are common in the palatine tonsils. The terms keratin cyst, used by the authors above, and marked crypt dilatation refer to the same type of change. Tan et al. (2018) suggest that these cysts may arise due to occlusion of the crypts during the embryonic period or surgery or trauma. In the cases of this study, these lesions were probably caused by trauma due to the ingestion of coarse vegetable fibers since it was not uncommon to find them in the lumen of the crypts (37.92%, 124/327).

The second most frequently observed change in the present study was hyperplasia of lymphoid follicles (304, 92.96%).

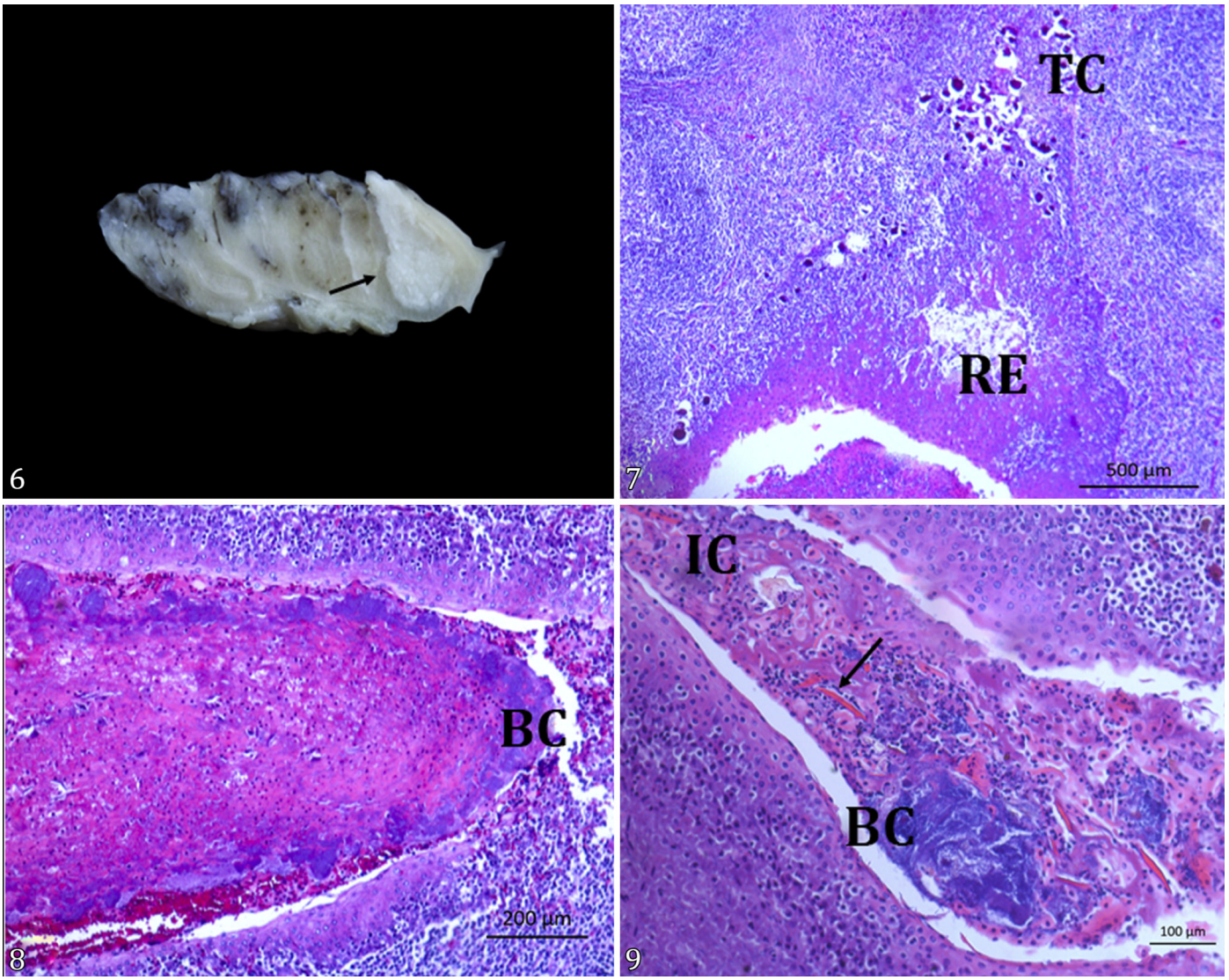

Fig.6-9. Pathological changes in palatine tonsils of buffaloes. Crypt abscesses. (**6**) Buffalo TO 280: Abscess filled with yellowish purulent exudate (arrow); the dried appearance of the exudate is due to the fixation of the specimen; in the fresh specimen, the consistency was pasty. (**7**) Buffalo TO 186: Crypt filled with inflammatory cells, area of lymphoepithelium or reticulation (RE) of the crypt epithelium with tonsillar corpuscles (TC) at the base of the epithelium and moderate lymphoid hyperplasia. HE, obj.5x. (**8**) Buffalo TO 202: Distended crypt with inflammatory cells and basophilic bacterial colonies (BC) on the surface. HE, obj.10x. (**9**) Buffalo TO 177: Crypt with numerous inflammatory cells (IC), basophilic bacterial colonies (CB) and plant fibers (arrow). HE, obj.20x.

Table 1. Pathological changes in palatine tonsils of slaughtered water buffaloes (*Bubalus bubalis*): Crypt dilatation/keratin cyst

Type of lesion	Intensity				Total
	Distribution	Mild	Moderate	Marked	
Crypt dilatation/cyst	Focal	3 (0.96%)	0	0	3 (0.96%)
	Multifocal	123 (39.17%)	119 (37.90%)	69 (21.97%)	311 (99.04%)
TOTAL		126 (49.13%)	119 (37.90%)	69 (21.97%)	314

Table 2. Pathological changes in palatine tonsils of slaughtered water buffaloes (*Bubalus bubalis*): Crypt abscess

Crypt abscess	Distribution	Intensity		Sum	
		Mild	Moderate	Changes	Percentage
With inflammatory cells only	Focal	4 (1.53%)	0	22	8.42
	Multifocal	17 (6.51%)	1 (0.38%)		
Inflammatory cells+bacteria	Focal	26 (9.96%)	4 (1.53%)	115	44.05
	Multifocal	55 (21.07%)	30 (11.49%)		
Inflammatory cells+plant fibers	Focal	2 (0.76%)	1 (0.38%)	12	6.12
	Multifocal	8 (3.06%)	1 (0.38%)		
Inflammatory cells+bacteria+plant fibers	Focal	12 (4.60%)	0	112	42.91
	Multifocal	62 (23.75%)	38 (14.56%)		
GRAND TOTAL OF CHANGES		186 (71.26%)	75 (28.72%)	261	

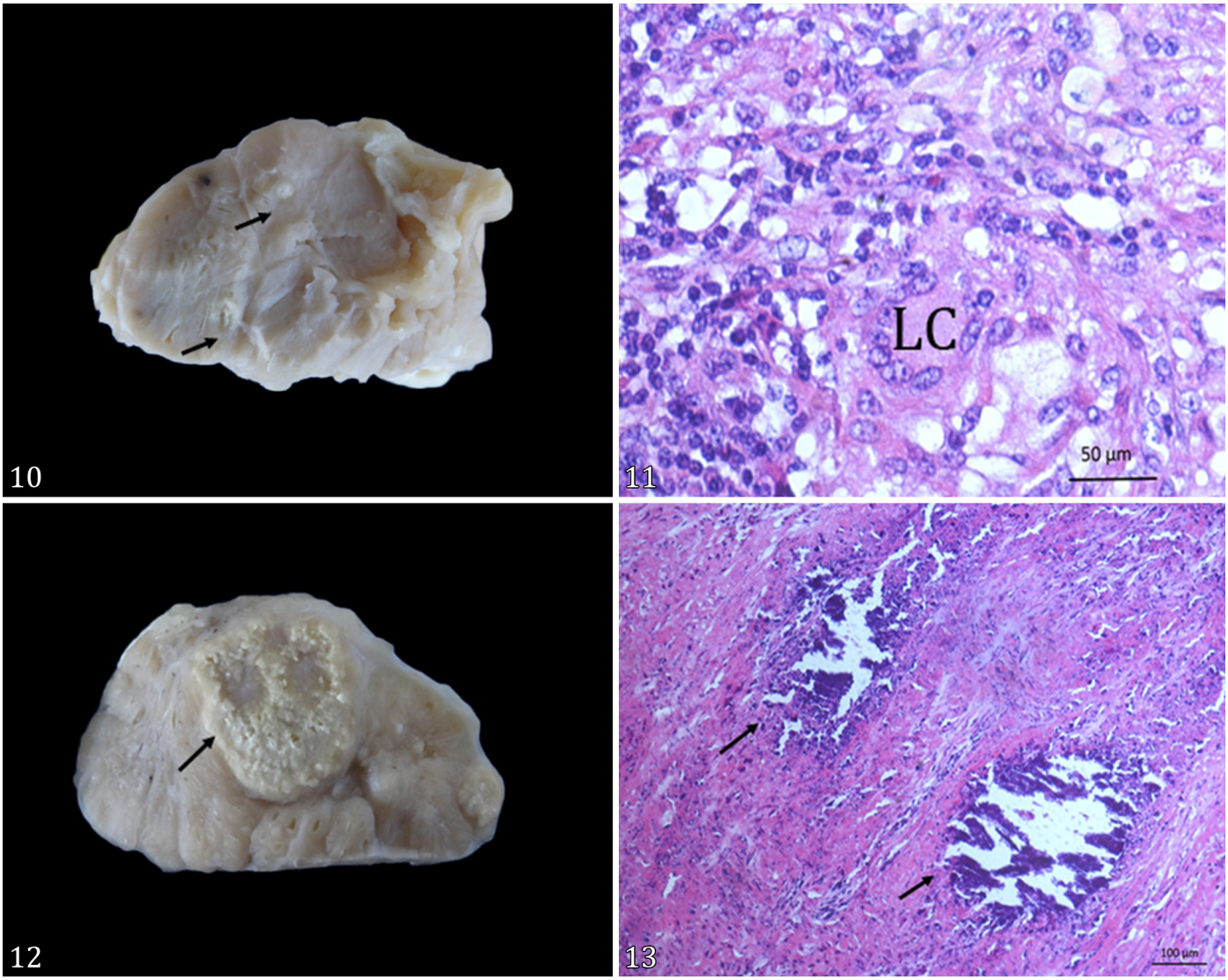

Fig.10-13. Pathological changes in palatine tonsils of buffaloes. Macroscopic and microscopic lesions compatible with tuberculosis. (**10**) Buffalo TO 75: Scattered foci of caseous necrosis (arrows). (**11**) Buffalo TO 75: Granulomatous inflammation with Langhans giant cells (LC), macrophages and lymphocytes in the parenchyma of the tonsil. HE, obj.40x. (**12**) Buffalo TO 167: Extensive area of caseous necrosis with mineralization (arrow). (**13**) Buffalo TO 167: Tonsillar parenchyma with extensive areas of caseous necrosis and basophilic foci of central mineralization (arrows). HE, obj.20x.

Some authors consider lymphoid follicle hyperplasia and reticular epithelium density of the crypts to be dynamic changes influenced by several factors, such as age and increased exposure to antigens (Perry & Whyte 1998, Girgiri & Kumar 2018, Sun et al. 2018, Tan et al. 2018). Thus, hyperplasia of lymphoid follicles in the tonsils is considered a common and nonspecific finding (Girgiri & Kumar 2018, Tan et al. 2018), which is corroborated by the data obtained in the present study. The peculiar characteristics of the tonsillar crypts of buffaloes (Zidan & Pabst 2011, Girgiri & Kumar 2021) favor the immune function in the species and, consequently, the high frequency of the follicular hyperplasia observed in this study. In some palatine tonsils in the study, follicle hyperplasia was associated with crypt abscesses.

Other frequent changes found herein were tonsillar crypt abscesses (79.82%, 261/327), with 71.26% (186/261) of these being considered mild and 28.74% (75/261) moderate. Similar lesions are also frequently observed in palatine tonsils of bovines (Cassidy et al. 1999) and humans (Wilkinson 1929, Tan et al. 2018). Wilkinson (1929) stated that all tonsils submitted to tonsillectomy evaluated in his study presented some level of leukocyte infiltration in the lumen. These crypt abscesses in humans can ulcerate the mucosa and extend into the parenchyma and peritonsillar tissues, especially when associated with viral infection (Tan et al. 2018). Nonetheless, this was not observed in the buffaloes in the current study, where inflammation was restricted to the lumen of the crypts. The tonsillar crypts are partially covered by reticular epithelium that allows for a greater interaction between epithelial and non-epithelial cells, which facilitates the contact of leukocytes with antigens present in the lumen of the crypts (Perry & Whyte 1998, Zidan & Pabst 2011). The apparently higher frequency of this finding in human-related studies is probably due to the evaluation of these tonsils

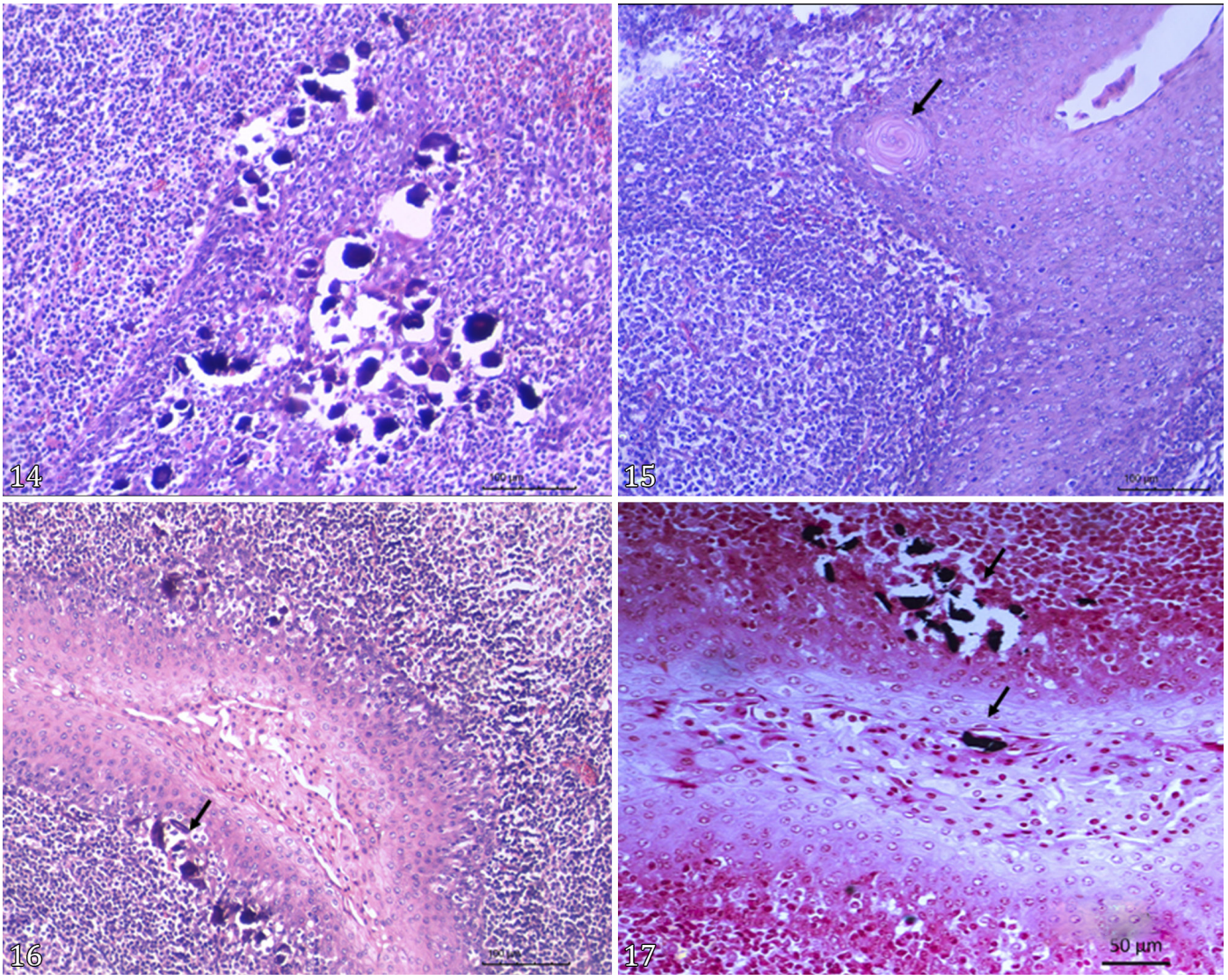

Fig.14-17. Pathological changes in palatine tonsils of buffaloes. Tonsillar corpuscles. (**14**) Buffalo TO 186: numerous tonsillar corpuscles in the basal layer of the crypt epithelium and the subepithelial lymphoid tissue (arrow). HE, obj.20x. (**15**) Buffalo TO 94: Keratin pearl (arrow) at the base of the crypt epithelium and hyperplastic lymphoid follicle. HE, obj.20x. (**16**) TO 186: Numerous corpuscles with a similar distribution to Figure 14. HE, obj.20x. (**17**) Buffalo 186: Presence of calcium salts in the tonsillar corpuscles (arrow). Von Kossa staining, obj.40x.

removed for clinical indications, such as cases of chronic or recurrent tonsillitis (Darrow & Siemens 2002). In contrast, this study evaluated buffaloes with no apparent clinical signs.

The overwhelming majority (91.57%, 239/261) of cases of tonsillar crypt abscesses in the buffaloes in the current study were associated with the presence of bacterial colonies and/or plant fibers in the lumen, which was also reported by Cassidy et al. (1999) in bovines. Most (88.83%) of the buffaloes in the current study came from Marajó Island, where they are raised almost exclusively on an extensive basis, with an exclusive diet of pasture, often fibrous, rich in lignin. Thus, it is possible that part of these changes is due to traumatic injuries that enable secondary colonization by bacteria. Another possibility is primary colonization by pathogens. The palatine tonsils can act as an entry point, latency site and maintenance site for various pathogens (De Vos et al. 2001, De Klerk et al. 2006, Annas et al. 2014); thus, future studies on the agents involved in this type of lesion in buffaloes are necessary.

Tonsillar corpuscles were observed in 31.19% of buffaloes' tonsils, and these structures have been reported in the tonsils of several species, such as humans, gorillas, dogs, pigs (Perry & Slípka 1993), horses, and goats, but not in buffaloes (Girgiri & Kumar 2018). Thus, this research demonstrates that these structures can also occur in the tonsils of Asian buffaloes. It was not possible to unequivocally explain the apparent high frequency of these bodies in the buffaloes in the present study. However, Sato et al. (1990) and Perry & Slípka (1993) report that these structures are related to keratinization and degeneration of basal layer epithelial cells as a result of increased lymphocyte transit in the reticular epithelium. These authors also mention that continuous antigenic stimulation can increase the number and size of the tonsillar corpuscles. The data contained herein corroborate this hypothesis.

In the analyzed tonsils, there were also granulomatous lesions compatible with tuberculosis in 1.53% (5/327). In four out of the five cases, there were similar lesions in other organs, which led to total condemnation of the carcass. In only one case, granulomatous lesions were seen only in the palatine tonsils. *Mycobacterium bovis* is the main agent isolated from tuberculosis lesions in buffaloes in the Brazilian Amazon (Freitas et al. 2001, Pedroso et al. 2021). Experimental studies, with intra-tonsillar inoculation of *M. bovis*, have demonstrated the viability of the palatine tonsils as the initial focus of tuberculosis lesions in cattle, deer (*Cervus elaphus*) (Griffin et al. 2006) and African buffaloes (*Syncerus caffer*) (De Klerk et al. 2006). Studies in cattle have also shown the development of tonsillar lesions from intranasal inoculation and contact with cattle infected with *M. bovis* (Cassidy et al. 1999). Tuberculosis lesions on the palatine tonsils may also occur through contact with sputum exudate from pulmonary tuberculosis lesions (Cassidy et al. 1999, Dhanasekar et al. 2020) or by the installation of the agent from the circulating blood (Dhanasekar et al. 2020). Thus, in the four cases in the current study, where, besides the palatine tonsils, there were lesions in other organs, it was not possible to state whether the tonsillar lesions were primary or secondary. However, in the case of this study, with lesions restricted to the palatine tonsils, this was most likely the initial focus of the disease. Tuberculosis cases restricted to the tonsils (tonsillar tuberculosis) are described in humans. Although now rare, this form of tuberculosis was most frequent when it was common to drink unpasteurized milk contaminated with *M. bovis* (Dhanasekar et al. 2020).

The palatine tonsils are situated in the drainage area of the retropharyngeal lymph nodes, which are frequently involved in tuberculosis in cattle (Cassidy et al. 1999) and buffaloes (Freitas et al. 2001). The present study indicates that the palatine tonsils are also important in the pathogenesis of tuberculosis in buffaloes. The data demonstrate that evaluating the palatine tonsils of buffaloes can contribute to increasing the possibility of detecting tuberculosis lesions in the sanitary inspection of buffaloes.

In the buffaloes in the current study, AFB was found in 20% (1/5) of the palatine tonsils with lesions compatible with tuberculosis. The literature points out that tuberculosis lesions in ruminants are generally paucibacillary; that is, they have small amounts of AFB. Pereira et al. (2017), studying tuberculosis lesions in buffaloes slaughtered for consumption in Amapá, found AFB in 15% (3/20) of the animals. Cassidy et al. (1999), when studying positive bovines in the tuberculization test in the slaughterhouse, observed tuberculous lesions in the palatine tonsils in 28.13% (9/32) and in 66.67% (6/9) of them AFB found. In a study evaluating two African buffaloes (*S. caffer*) positive for tuberculosis on interferon-ɣ examination, no AFB was observed in the lesions (Garine-Wichatitsky et al. 2010). Another study in African buffaloes found AFB in 36.8% (7/19) of animals with granulomatous lesions (Laisse et al. 2011). The identification of AFB is important for the histopathological diagnosis of tuberculosis; however, it is well-established that mycobacteria are the main cause of necrotizing granulomas, such as those observed in our study (Shah et al. 2017). Pedroso et al. (2021) also report a good agreement between histopathological analysis and molecular detection of *Mycobacterium* spp. in bubalines.

CONCLUSIONS

The data in the current study demonstrate that evaluating the palatine tonsils of buffaloes at the slaughterhouse can provide important information about subclinical or asymptomatic tonsillitis. There is a high frequency of tonsillar crypt abscesses associated with the presence of bacteria and/or plant fibers, which requires further etiological studies.

The observation of tuberculosis lesions in the palatine tonsils of buffaloes indicates that these may contribute to the pathogenesis of the disease in this species as well. The evaluation of the palatine tonsils can increase the possibility of identifying tuberculosis cases in the sanitary inspection. Dilatation of tonsillar crypts, keratin cysts, follicular hyperplasia and tonsillar corpuscles are frequent findings in the palatine tonsils of buffaloes.

The tonsillar lesions in buffaloes without apparent clinical signs described herein indicate the need for additional etiological studies in palatine tonsils of bubalines aiming at bacterial and/or viral agents in reasonable equilibrium with the animals' organism.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

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