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Case Report - Veterinary Medicine

Bilateral posterior ocular segment coloboma, entropion and squamous cell carcinoma in buffaloes with oculocutaneous albinism: a clinical, ultrasound, microbiological, cytological, and histopathological evaluation

[*Coloboma bilateral de segmento posterior, entrópio e carcinoma de células escamosas em búfalos com albinismo oculocutâneo: avaliação clínica, ultrassonográfica, microbiológica, citológica e histopatológica*]

A.M.V. *Lima*[1], A.R. *Fayad*[1], B.M. *Assis*[2], C.A. *Neves*[1], N.C. *Borges*[1], F. *Camargo*[1], D.B. *Martins*[1], C.H.S. *Oliveira*[3], E. *Perlmann*[4], P.J.B. *Queiroz*[1], R.E. *Rabelo*[5], L.A.F. *Silva*[1]

[1]Universidade Federal de Goiás, Goiânia, GO, Brasil
[2]Faculdade UNA- Unidade Jataí, GO, Brasil
[3]Ministério da Agricultura e Pecuária, Goiânia, GO, Brasil
[4]Practitioner, São Paulo, SP, Brasil
[5]Practitioner, Jataí, GO, Brasil

ABSTRACT

The study of ocular lesions associated with oculocutaneous albinism (OCA) in buffalo contributes to understanding the impact of this condition on buffalo production. The aim of this article is to describe the multiple ocular alterations observed in two buffaloes with OCA. A 13-month-old Mediterranean-Jafarabadi male and an 8-year-old Mediterranean female underwent ophthalmic, ultrasound, microbiological, cytological and histopathological evaluation. The male showed entropion and coloboma of the retina, choroid, and sclera. The female presented a proliferation of neoplastic cells consistent with squamous cell carcinoma in the bulbar conjunctiva and third eyelid. Both animals had a Schirmer tear test between 25 and 35mm/min and intraocular pressure between 11 and 15mmHg. There was growth of *Pseudomonas maltophilia*, *Enterobacter aerogenes*, and *Escherichia coli* and *Rhodotorula* sp. in both samples collected from the conjunctival fornices. A variable number of epithelial cells, lymphocytes and neutrophils were identified by conjunctival cytology. No ultrasound changes were identified. In view of the findings, a complete ophthalmic examination of buffaloes with OCA is recommended as a way of investigating alterations that are not always obvious on remote inspection, as well as avoiding exposure to solar radiation to minimize the occurrence of ocular squamous cell carcinoma.

Keywords: buffalo, albinoid, eye, coloboma, carcinoma

RESUMO

O estudo das lesões oculares em búfalos com albinismo oculocutâneo (OCA) contribui para conhecer o impacto dessa condição na produção de bubalinos. Este artigo tem por objetivo descrever alterações oculares múltiplas observadas em dois búfalos portadores de OCA. Um macho mestiço (Mediterrâneo e Jafarabadi), de 13 meses de idade, e uma fêmea da raça Mediterrâneo, de 8 anos de idade, foram submetidos à avaliação oftálmica, ultrassonográfica, microbiológica, citológica e histopatológica. O macho apresentou entrópio e coloboma de retina, coroide e esclera. Na fêmea, foi identificada proliferação de células neoplásicas condizente com carcinoma de células escamosas na conjuntiva bulbar e na terceira pálpebra. Ambos apresentaram teste lacrimal de Schirmer entre 25mm/min e 35mm/min, e pressão intraocular entre 11mmHg e 15mmHg. Houve crescimento de Pseudomonas maltophilia, Enterobacter aerogenes, E. coli *e* Rhodotorula *sp. em ambas as amostras colhidas dos fórnices conjuntivais. Identificou-se, pela citologia conjuntival variável, número de células epiteliais, linfócitos e neutrófilos. Não foram identificadas alterações ultrassonográficas. Diante dos achados, recomenda-se o exame oftálmico completo de búfalos com OCA como forma de investigação de*

Corresponding author: aline_lima@ufg.br
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alterações nem sempre óbvias na inspeção a distância, assim como a prevenção da exposição à radiação solar para minimizar a ocorrência de carcinoma de células escamosas.

Palavras-chave: bubalino, albinoide, olho, coloboma, carcinoma

INTRODUCTION

Oculocutaneous albinism (OCA) has been commonly observed in buffaloes in Brazil and is described as a likely result of inbreeding, as the national herd originated from just over 200 imported animals (Damé *et al.*, 2015; Bernardino *et al.*, 2019). Buffaloes with little or no pigmentation are more susceptible to ocular diseases (Damé *et al.*, 2015), which negatively affect productivity, making partial or total albinism an undesirable characteristic (Bernardino *et al.*, 2019).

Studies aimed at ophthalmology in buffaloes, including reports of ophthalmopathies and ocular morphophysiological characteristics, have been described in recent years (Di Pietro *et al.*, 2012; Assadnassab and Fartashvand, 2013; Sivaseelan *et al.*, 2008; Lamagma *et al.*, 2015; Kuma and Sharif, 2018). However, there are few reports describing ocular diseases in buffaloes with OCA (Damé *et al.*, 2015), and no studies evaluating morphophysiological characteristics of the eyes of albinoid specimens were found in the consulted literature. In this context, this study aimed to report ocular disorders observed in two albinoid buffaloes, as well as describe clinical, ultrasound, cytological, histological, and microbiological ocular parameters in these specimens.

CASE REPORT

An ophthalmic evaluation was carried out on two albinoid buffaloes, a 13-month-old Mediterranean and Jafarabadi mixed breed male and an 8-year-old Mediterranean female, both weighing approximately 450 kg. The animals were restrained in a chute and had their heads positioned with the aid of ropes to carry out the examinations. The right and left eyes of both animals were subjected to clinical ophthalmic evaluation, conjunctival sample collection for culture and cytology, retinography, and ocular ultrasound. The buffalo were humanely slaughtered after being stunned in captivity in a commercial slaughterhouse due to the end of the animals' productive cycle. After slaughtering the two eye bulbs were collected for histopathological examination.

The ophthalmic clinical evaluation consisted of a visual field test, pupillary light reflex, Schirmer tear test (Drogavet®, Curitiba, PR, Brazil), fluorescein test (Sodium Fluorescein 1%®, Allergan Inc®, Guarulhos, SP, Brazil), applanation tonometry (Tonopen®, Reichert Technologies, New York, NY, United States) under local anesthesia (Anestalcon®, Alcon, São Paulo, SP, Brazil), inspection of ocular adnexa, slit lamp biomicroscopy in the cornea, anterior chamber, iris, and pupil; slit lamp biomicroscopy in the lens and vitreous under pharmacological mydriasis (Mydriacil®, Alcon, São Paulo, SP, Brazil); monocular indirect ophthalmoscopy (Volk®, Ohio, OH, United States); and retinography (Clearview Fundus Camera, Optibrand®, Fort Collins, USA).

The conjunctival sample for culture was collected immediately after the Schirmer tear test. Two samples were taken from each eye by using a sterile swab from the lower conjunctival fornix for bacterial and fungal cultures. The bacterial culture was performed on blood agar and McConkey agar for up to 72 hours. Colonies were identified with biochemical tests. The fungi were cultivated on Sabouraud agar medium and identified by morphology.

An interdental brush (Bitufo® HBA 3mm) was gently rubbed four times on the lower conjunctival fornix in a rotating movement and rolled over identified glass slides to collect a conjunctival sample for cytology. The slides were dried at room temperature and then fixed in methanol and, subsequently, stained with rapid panoptic. The cytological evaluation was carried out using a 10x objective to identify areas with a greater distribution of cells and 40x for identification and morphological characterization of cells. The counting and differentiation of cell types were carried out by counting 200 cells in a tower movement.

A drop of anesthetic eye drops was then instilled again and transcorneal ultrasound was performed

using a Logic E ultrasound device (GE Healthcare®, Waukesha, WI, United States) coupled to a linear transducer with a 12 MHz frequency. A sterile acoustic gel was used, and the transducer was protected with a layer of gel covered with cling film (Lusafilm R 105®, Guarulhos, SP, Brazil).

A biopsy of the conjunctiva of the third eyelid was performed on the female's left eye due to tissue proliferation noticeable during the ophthalmic examination. Tissue was collected at the end of all described evaluations. After slaughtering the animals, the eye bulbs were collected, fixed in 10% buffered formalin, subjected to routine histotechnical processing using hematoxylin and eosin staining, and evaluated under an optical microscope. The eyes were subjected to macroscopic and microscopic evaluation.

Positive visual field and pupillary light reflexes were observed in both animals as a result of the evaluations. The male showed upper eyelid entropion, trichiasis, and bilateral palpebral conjunctival hyperemia (Figs. 1A and 1B), STT of 35mm/min in the RE and 30 mm/min in the LE, negative fluorescein test, and IOP of 13mmHg in the RE and 15 mmHg in the LE. The female presented mucopurulent ocular discharge in the left eye and the presence of pinkish proliferative tissue on the palpebral surface of the third eyelid, STT of 35mm/min in the RE and 25mm/min in the LE, negative fluorescein test, and IOP of 11 mmHg in the RE and LE. No changes were observed in the cornea, sclera, anterior chamber, iris, lens, and vitreous for both specimens. Ophthalmoscopy and retinography of both buffaloes revealed the presence of a red fundus reflex, *tapetum lucidum*, and pigmented areas. Changes suggestive of coloboma of the retina, choroid, and sclera were observed in the ocular fundus of the male's right and left eyes, characterized by irregularity of the edge of the optic papilla and a circular area of excavation with a whitish fundus, which covered the peripapillary areas (Figs. 1C and 1D).

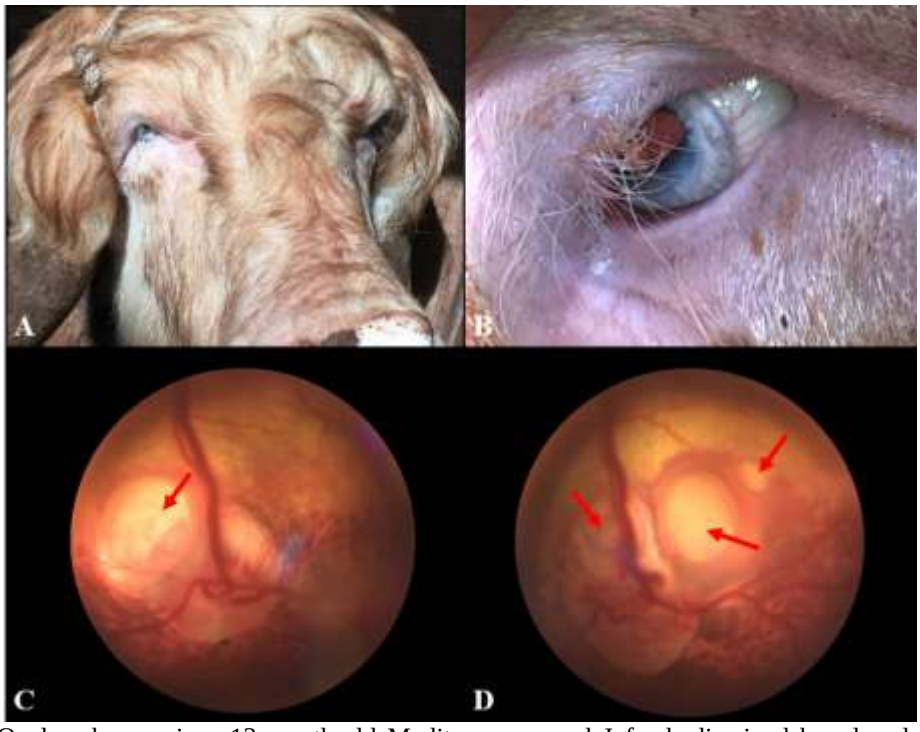

Figure 1. Ocular changes in a 13-month-old Mediterranean and Jafarabadi mixed breed male albinoid buffalo. (A) Total depigmentation of the right eyelids and iris and partial depigmentation of the nostril. (B) Right eye with upper eyelid entropion and trichiasis. (C and D) Retinography showing coloboma as whitish areas of discontinuity of the papillary edge with excavation (arrows) in the right (C) and left (D) eyes.

No changes were observed in the ultrasound images in the studied animals. Table 1 shows the results of the microbiological and cytological examinations of the conjunctiva. The histological evaluation of the mass on the female's left third eyelid showed changes compatible with squamous cell carcinoma. Neoplastica epithelial cells, presenting loss of epithelial architecture, moderate atipia and pleomorphism and stromal invasion were also observed in the bulbar conjunctiva of the left eye of the same animal.

Table 1. Result of bacterial and fungal cultures and conjunctival cytopathology (absolute number and percentage of cells) of the right (RE) and left (LE) eyes of the animals

Parameters	Male RE	Male LE	Female RE	Female LE
Bacterial culture	*Pseudomonas maltophilia*	*Pseudomonas maltophilia*	*Enterobacter aerogenes; E. coli*	*Enterobacter aerogenes; E. coli*
Fungal culture	*Rhodotorula sp.*	-	-	*Rhodotorula sp.*
Basal cells	5 (2.5%)	7 (3.5%)	15 (7.5%)	18 (9%)
Intermediate cells	85 (42.5%)	136 (68%)	112 (56%)	100 (50%)
Superficial cells	36 (18%)	42 (21%)	22 (11%)	42 (21%)
Keratinized cells	3 (1.5%)	0 (0%)	2 (1%)	2 (1%)
Globet cells	2 (1%)	0 (0%)	0 (0%)	2 (1%)
Lymphocytes	69 (34.5%)	15 (7.5%)	37 (18.5%)	29 (14.5%)
Neutrophils	0 (0%)	0 (0%)	12 (6%)	7 (3.5%)
Red blood cells	-	-	+	-

The anatomopathological examination of the male's ocular bulbs confirmed the suspicion of coloboma, characterized as a peripapillary depression with a whitish appearance and absence of vessels on the macroscopic evaluation (Figs. 2A and 2B), and a defect in the posterior wall with loss of continuity of the sclera/choroid/retina and formation of a scleral depression in the peripapillary region on the microscopy evaluation (Fig. 2C). Furthermore, the left eye showed this filling of this space with retinal nerve fibers (Fig. 2D).

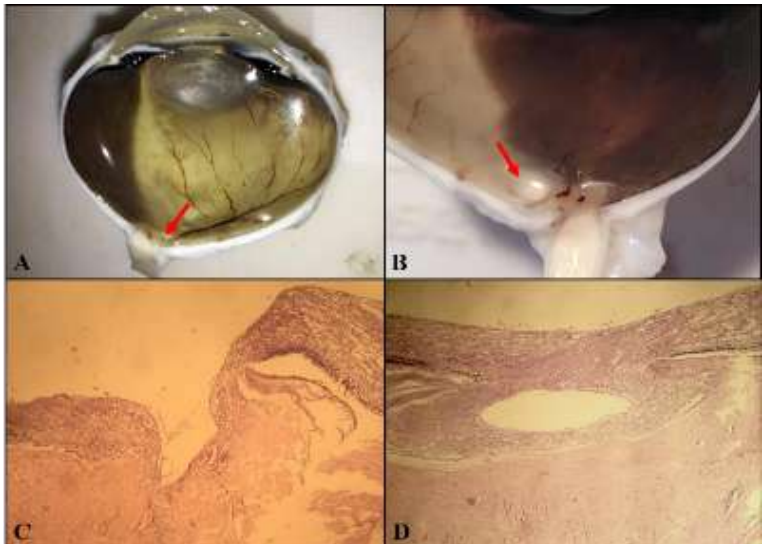

Figure 2. Coloboma of the retina, choroid, and sclera in a 13-month-old Mediterranean and Jafarabadi mixed breed male albinoid buffalo. (A and B) Longitudinal section of the ocular bulbs fixed in formalin with evidence of coloboma due to the depression of the scleral tissue in (A) (arrow) and excavation in the peripapillary area in (B) (arrow). (C and D) Ocular histopathology showing a defect in the posterior wall with loss of continuity of retina, choroid, and sclera in (C) and a scleral defect filled by retinal nerve fibers in (D). HE, 100x magnification.

DISCUSSION

The phenotypic characteristics of oculocutaneous albinism (OCA) observed in the buffaloes studied consisted of partial or complete depigmentation of the mucocutaneous regions, nasal planum, iris and coat, and are the result of the partial or total absence of melanin in these structures, described by Damé *et al*. (2012) as an autosomal recessive hereditary disorder that leads to a deficiency of the tyrosinase enzyme.

Although cases of OCA in buffalo have been described in Brazil, mostly in the Murrah breed (Damé *et al*., 2013), to the authors' knowledge, this is the first report of ocular coloboma of the posterior segment in albino buffalo. Colobomas are congenital defects of the ocular tissues and most commonly involve the iris, choroid, sclera and optic papilla (Cook, 2021). The retinography and histopathology images obtained from the buffalo under study are compatible with the description of retinal, choroidal and scleral coloboma for albinoid cattle (Martins, 2021).

The colobomas observed in the buffalo under study had a peripapillary location and can be defined as atypical colobomas. Unlike the typical coloboma, which results from failure to close the optic fissure during embryonic development and is located at the 6h position of the iris, choroid, sclera, and/or optic papilla (Cook, 2021); the atypical coloboma in albino buffalo may result from a defect in the pigmentation of the retinal pigmented epithelium (RPE) which compromises the differentiation of structures in the posterior ocular wall (Cook, 2021). Corroborating this hypothesis, a similar spectrum has been identified in cattle (Gelatt *et al*., 1969), Great Dane dogs and cats (Gwin *et al*., 1981) with incomplete albinism.

The colobomas identified on ophthalmoscopy and retinography were not visible at ultrasound examination. Ultrasound evaluation of coloboma is expected to show discontinuity in the posterior ocular wall with a focal oblong anechoic lesion on the posterior aspect of the globe (Venincasa *et al*., 2015; Moore and Lamb, 2007). In the described case, histopathological analysis showed total discontinuity of the retinal and choroidal tissues, but only partial discontinuity of the scleral tissue, which maintained the fibrous structure of the posterior ocular wall, justifying the non-observation of the defect by ultrasound.

Although entropion has already been described in cattle (Martins, 2021), no reports in buffaloes were found in the consulted literature. In the case study, the eyelid inversion was associated with trichiasis and conjunctival hyperemia. Chronic inflammation of the conjunctiva secondary to entropion may justify the observation of inflammatory cells, predominantly lymphocytes, in the cytological evaluation.

It is believed that the development of squamous cell carcinoma in the ocular conjunctiva of the female studied is related to the lack of pigmentation and chronic exposure to solar radiation in the tropical climate region in which the animals lived. In agreement, melanoma and squamous cell carcinoma have already been related to OCA in cattle (Damé *et al*., 2015), and both neoplasms are associated with chronic exposure of depigmented areas to solar radiation.

The STT values found in both eyes of the male and in the left eye of the female were higher than those observed in a study of 40 buffaloes in Italy, which were 22.37 ± 5.44 mm/min (Di Pietro *et al*., 2012). This probably reflects the tearing secondary to entropion, trichiasis and conjunctivitis observed in the male and the irritative factor associated with depigmentation and neoplastic proliferation on the female's ocular surface. IOP also differed, with lower values in the buffaloes studied compared to the aforementioned study (20.12±3.44mmHg). Reduced IOP values are often associated with uveitis, but in the two buffaloes studied, no other clinical signs or histological changes were found to support the diagnosis of a uveal inflammatory process. Importantly, different ways of restraining the animals, evaluation times, evaluators, and tools can be factors that influence IOP measurement.

Transcorneal ocular ultrasound was performed to visualize the coloboma in the imaging tool, however in this study it was not possible to visualize the malformation using this exam. Additionally, ocular ultrasound demonstrated to be a feasible technique for buffaloes, and the ocular structures evaluated using ultrasound showed echogenicity, echotexture, and echobiometry similar to other studies in

buffaloes (Kassab, 2012; Assadnassab and Fartashvand, 2013).

The conjunctival cytology of both animals revealed a predominance of intermediate cells, followed by basal, superficial, keratinized superficial cells, and goblet cells. Lamagma *et al*. (2015) used the same methodology and observed the predominance of basal cells in the conjunctival cytology of buffaloes. The authors also described the inflammatory cells found in conjunctival cytology, with a higher proportion of neutrophils (70%) compared to lymphocytes (10%), differing from the results observed in the two studied buffaloes, in which there was a marked predominance of lymphocytes compared to neutrophils. The lymphocytic inflammatory profile is related to chronic inflammation, which may be associated with entropion and tissue sensitivity to ultraviolet radiation.

In this case, there was the growth of *Pseudomonas maltophilia* in the conjunctival bacterial culture of the male and *Enterobacter aerogenes* and *E. coli* of the female, which are, in all cases, gram-negative bacteria. A study carried out on 57 healthy buffaloes showed a 69.95% frequency of gram-positive bacteria, predominantly *Staphylococcus lentus* and *Enterococcus faecium*, and a 30.65% frequency of gram-negative bacteria, predominantly *E. coli* (Lamagma *et al*., 2015). The isolation of these bacteria does not seem to have clinical relevance for the buffaloes studied, and they may probably be part of the conjunctival microbiota of these animals.

The yeasts of *Rhodotorula sp.*, isolated from the conjunctiva of the male's right eye and the female's left eye, were not described as part of the conjunctival microbiota of buffaloes by Lamagma *et al*. (2015). According to Khosravi *et al*. (2014), isolation of *Rhodotorula* in conjunctival samples can be result of water contamination and poor bedding quality and may reflect high presence of these fungi in their habitat. Despite its presence in the conjunctiva, it did not appear to be related to the changes found in the buffaloes evaluated. The characterization of the ocular microbiota of buffaloes is relevant because the animals can be potential vectors of bacteria that can be resistant to antimicrobials. Moreover, it may help in the therapeutic choice of ocular surface diseases and the discernment between microbiota and pathogens.

In this study, the authors demonstrated the occurrence of congenital and acquired eye conditions in buffaloes with OCA through detailed ophthalmic examination. In addition, it was possible to investigate morphology and microbiology and associate them with the ophthalmopathies observed. Among the limitations, although case reports provide important information, it does not allow the findings to be extrapolated to the species. Further studies in buffaloes, with and without OCA, in specific geographical locations and management conditions, could contribute to our knowledge of ophthalmic parameters and diseases, as well as the cytological and microbiological characteristics of the ocular surface in these animals.

CONCLUSION

The ocular examination in buffaloes with OCA provides information about ocular changes not always obvious on remote inspection, like entropion and colobomas. Ocular fundus exams can be helpful to diagnose malformations in albinoid buffaloes. Care must be done to prevent solar radiation exposure and minimize the occurrence of squamous cell carcinoma.

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