

# Adenoma of the *pars intermedia* of the pituitary gland in a horse in Colombia, morphological and immunohistochemical study: Case report

*Adenoma de la pars intermedia de la hipófisis en un caballo en Colombia, estudio morfológico e inmunohistoquímico: Reporte de caso*

*Adenoma da pars intermedia da glândula pituitária em cavalo na Colômbia, estudo morfológico e imuno-histoquímico: Relato de caso*

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## Abstract

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**Anamnesis:** An Argentine Saddle Horse with poor body condition, general weakness, and recurrent pathological decubitus that did not respond to treatment was euthanized and subjected to diagnosis. **Clinical and laboratory findings:** At necropsy, hirsutism and poor body condition were observed. The pituitary gland protruded from the sella turcica and measured 2.3 cm in thickness and 2.8 cm in high. A nodule measuring 1.3 cm in diameter was observed in the *pars intermedia* of the pituitary gland. Histological findings revealed an adenoma in the *pars intermedia* of the pituitary gland. The neoplastic cells were immunoreactive for ACTH, and the markers for PRL, NSE, and GFAP were negative. The ultrastructure of the neoplastic cells showed intracytoplasmic granules of variable electron density with diameters between 160 and 250 nm. **Conclusion:** This is the first report of a *pars intermedia* adenoma in an Argentine saddle horse and the first comprehensive diagnosis of this neoplasia in Colombia and South America.

**Keywords:** ACTH; Argentine Saddle Horse; Colombia; equine; gelded male; neoplasia; pituitary.

## Resumen

**Anamnesis:** Un Caballo Silla Argentina con mala condición corporal, debilidad general y decúbito patológico recurrente sin respuesta al tratamiento fue sacrificado y sometido a diagnóstico. **Hallazgos clínicos y de laboratorio:** En la necropsia se observó hirsutismo y mala condición corporal. La glándula pituitaria protruyó de la silla turca y medía 2,3 cm de grosor y 2,8 cm de alto. En la *pars intermedia* de la hipófisis fue observado un nódulo de 1,3 cm de diámetro. Los hallazgos histopatológicos revelaron un adenoma en la *pars intermedia* de la glándula pituitaria. Las células neoplásicas fueron inmunorreactivas para ACTH, mientras que los marcadores

para PRL, NSE y GFAP fueron negativos. La ultraestructura de las células neoplásicas mostró gránulos intracitoplasmáticos de densidad electrónica variable con diámetros entre 160 y 250 nm. **Conclusión:** Este es el primer reporte de un adenoma de *pars intermedia* en un Caballo Silla Argentina y el primer diagnóstico integral de esta neoplasia en Colombia y Sudamérica.

**Palabras clave:** ACTH; Caballo Silla Argentina; Colombia; equino; macho castrado; neoplasia; pituitaria.

## Resumo

**Anamnese:** Um Cavalo Sela Argentino com baixa condição corporal, fraqueza geral, decúbito patológico recorrente e sem resposta ao tratamento foi submetido à eutanásia e ao diagnóstico. **Achados clínicos e laboratoriais:** Na necropsia foram observados hirsutismo e baixa condição corporal. A glândula pituitária projetava-se da sela túrcica e medía 2,3 cm de espessura e 2,8 cm de altura e projetava-se da sela túrcica. Na *pars intermedia* da glândula pituitária observou-se um nódulo de 1,3 cm de diâmetro. Os achados histopatológicos revelaram um adenoma na *pars intermedia* da glândula pituitária. As células neoplásicas foram imunorreativas para ACTH e os marcadores de PRL, NSE e GFAP foram negativos. Na ultraestrutura das células neoplásicas foram observados grânulos intracitoplasmáticos de densidade eletrônica variável com diâmetros entre 160 e 250 nm. **Conclusão:** Este é o primeiro relato de um adenoma na *pars intermedia* em um Cavalo Sela Argentino e o primeiro diagnóstico abrangente desta neoplasia na Colômbia e na América do Sul.

**Palavras-chave:** ACTH; Cavalo Sela Argentino; Colômbia; equino; macho castrado; neoplasia; hipófise.

## Introduction

The adenoma of the *pars intermedia* (PI) of the pituitary gland is a frequently reported neoplasm in horses (Miller et al., 2016). The location of the PI adenoma hampers its surgical approach, and it is usually a cause of death or leads to compassionate euthanasia (Miller et al., 2016). When the adenoma is an endocrinologically active neoplasm, it is one of the recognized causes of the condition known as pituitary *pars intermedia* dysfunction (PPID) (Kirkwood et al., 2022). Degenerative diseases of the hypothalamic neurons contribute to loss of dopaminergic inhibition and are a risk factor for neoplasms of the *pars intermedia* (Fortin et al., 2021; Gris et al., 2023) producing proopiomelanocortin (POMC) and its derivatives, followed by growth alterations in the cells in the PI cells of the pituitary that protrude and compress the hypothalamus (McFarlane, 2007, 2011; Miller et al., 2016). The cause of neuronal degeneration is unknown; however, it has been proposed that the accumulation of misfolded alpha-synuclein may lead to such degeneration and hypothalamic compression, a mechanism similar to that described in Parkinson's disease

(Fortin et al., 2021). Other authors propose that excess production of POMC causes hyperplasia, hypertrophy, or adenomas in the PI (McFarlane, 2011; Fortin et al., 2021).

Immunohistochemistry (IHC) studies in equine endocrine neoplasia are scarce. Immunostaining of PI adenoma is usually positive and strong for POMC,  $\beta$ -endorphin ( $\beta$ -END),  $\alpha$ -melanocyte stimulating hormone (MSH) and adrenocorticotrophic hormone (ACTH) (Boujon et al., 1993; Meuten, 2017). Ultrastructural studies of neoplastic cells identify secretory granules, well-developed endoplasmic reticulum, and the accumulation of filaments and other organelles (Boujon et al., 1993).

To the authors' knowledge, there are no reports of pituitary adenomas in horses in Colombia and PI adenoma has not been described in Argentine Saddle Horses. Therefore, the aim of this study was to describe an adenoma of the PI of the pituitary in an Argentine Saddle Horse in Colombia, through a comprehensive study of the macroscopic lesions, optical microscopy, IHC and transmission electron microscopy (TEM).

## Case presentation

### Anamnesis

A horse was found in sternal recumbency and unable to stand in a paddock located in the Bogotá Savannah (North latitude 4,653, West longitude 74,097). The veterinarian reported that the horse had shown similar recurrent clinical signs over a period of approximately two years. Over time, the episodes became more frequent and the pathological prostration lasted longer. The horse's health deteriorated further in the last three months. The horses grazed on *Cenchrus clandestinum* (Kikuyu grass), supplemented with two rations of 1.5 kg per day of commercial feed for adult horses (Campeón® concentrate), mineralized salt and water *ad libitum*. Only one affected animal was found among a batch of 57 horses in total.

### Clinical findings and diagnostic aids used

The veterinarian reported that the clinical examination of the Argentine Saddle Horse, a 32-year-old gelding, revealed general body weakness, lethargy, and inability to stand. Intramuscular Phenylbutazone® (4.4 mg/kg SID) was administered, but there was no response to the treatment. Due to the animal's deterioration in recent months and the poor prognosis, the horse was euthanized on compassionate grounds. The body was sent for veterinary diagnosis to the Laboratorio de Patología Veterinaria de la Universidad Nacional de Colombia (LPV-UNAL).

At the necropsy, the animal had a poor body condition characterized by obvious bony protuberances, severe diffuse muscle atrophy, and scarce subcutaneous, mesenteric, and coronary sulcus adipose reserves. The palpebral and conjunctival mucosa were pale and moderately jaundiced. The presence of a long non-shedding hair coat was interpreted as hypertrichosis (hirsutism) (Fig. 1a). Thin, whitish, flaky material of different sizes and shapes was noted on the hair coat (dandruff). The pituitary gland was enlarged and protruded from the sella turcica (Fig. 1b). The size of the pituitary gland was 2.3 cm in thickness and 2.8

cm in height, with nodular areas. In the sagittal section, yellow nodules with brown foci were observed in the middle region. The diameter of the largest nodule was 1.3 cm (Fig. 1c).

The coronary, mesenteric and omental fat was scant, gelatinous in appearance, brownish-yellow in color, and translucent (reminiscent of adipose tissue serous atrophy). The thyroid glands showed moderate hypertrophy with a lobular appearance. When cut, abundant mucous-like content with a black color was discharged.

Samples of the pituitary gland, skin, thyroid gland, intestine, liver, and adrenal glands were collected for histology and fixed in buffered formalin (pH 7) for 48 h, for subsequent routine histological processing using the hematoxylin and eosin (H&E) technique.



Figure 1A

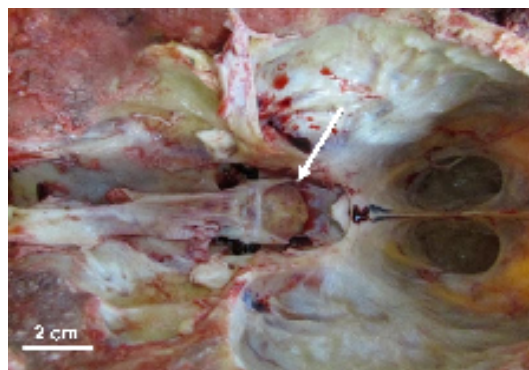


Figure 1B

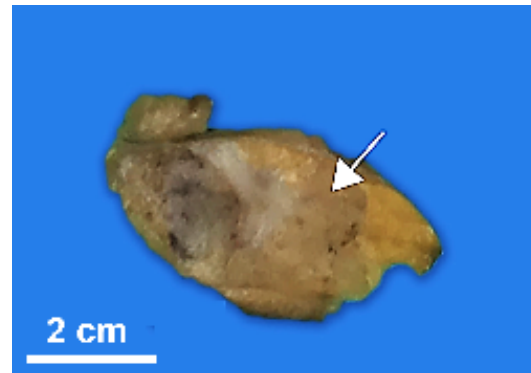


Figure 1C

**Figure 1. A.** Argentine Saddle Horse with adenoma of the *pars intermedia*. The horse had poor body condition and hirsutism. **B.** Exposed pituitary gland in the cranial cavity. The enlarged pituitary gland had nodules of firm consistency that protruded from the sella turcica (arrow). **C.** Sagittal section of the pituitary gland. The middle region of the pituitary revealed well-defined yellow nodules with brown spots, which displaced adjacent tissues (arrow) and altered the anatomical surface.

Microscopically, the PI of the pituitary gland had a partially encapsulated growth pattern that displaced and compressed the *pars distalis* and the neurohypophysis. The neoplastic cells were arranged in multiple nodules, cords and nests in 60% of the neoplasm (Fig. 2a), in 30% they formed pseudorosettes (radiated growth or palisade around blood vessels) (Fig. 2b) and 10% of the cells were organized around a colloidal-looking fluid content, arranged as thyroid follicle-like structures of different sizes and shapes with random distribution (Fig. 2c). The neoplastic cells were predominantly round, polyhedral and a few were spindle-shaped. The cytoplasm was granular, large, eosinophilic, and microvacuolated; some cells were binucleated. There was mild anisocytosis, mild pleomorphism, and mild cytomegaly with indistinguishable cell borders. The nuclei were large and chromatic, found in a central or parabasal position, round, oval, and a few had angular edges. Some nuclei showed mild pleomorphism, with cleft nuclei, a few aberrant nuclei, mild anisokaryosis, mild karyomegaly, vesiculated nuclei, fine granular chromatin, a prominent nucleolus, or two small nucleoli. Four mitoses were observed in 2.37 mm<sup>2</sup>.

Severe diffuse epidermal atrophy and

hyperplasia of hair follicles without sebaceous glands were observed in the skin, with most follicles in the anagen phase and a few in the catagen phase. Neurons at the brain base revealed central chromatolysis, perineural edema, neuronal retraction, and moderate satellitosis. In the adrenal gland, there was moderate diffuse cortical hyperplasia with hyperplastic capsule nodules. In the intestinal tract, mild diffuse neutrophilic fibrinous necrotic enterocolitis, with a few pseudomembranes and multiple bacterial colonies showing an intralesional coccobacilli morphology were observed. In the liver, severe active chronic random multifocal pyogranulomatous hepatitis with portal fibrosis and subacute severe diffuse suppurative cholangiohepatitis was observed.

Sequential sections for immunohistochemistry (IHC) studies were prepared from the pituitary tissue embedded in paraffin blocks. The tissues were deparaffinized and incubated with specific antibodies for the detection of pituitary hormones, followed by a HRP conjugated polymer detection. The IHC procedures were performed by the company INMUNOTECH®. The analysis was conducted by a trained veterinary pathologist from LPV-UNAL. The criteria for IHC evaluation were those described in the literature



(Boujon et al., 1993) with some modifications: (1) negative, without labeling; (2) Positive, when it revealed granular intracytoplasmic and/or perinuclear immunostaining. Positivity was classified as weak when immunostaining intensity was low in < 20% of neoplastic cells, moderate when affecting 20% - 50%, and strong when immunostaining was intense and well defined in > 50% of neoplastic cells. Each

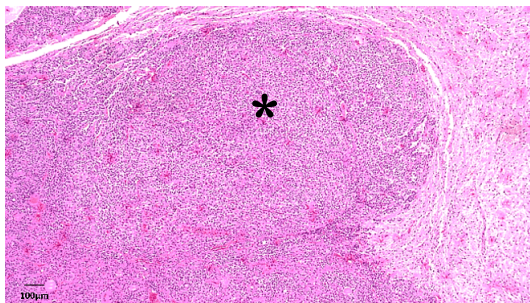
antibody had a positive control.

Neoplastic cells of the pituitary PI revealed strong intracytoplasmic and perinuclear granular ACTH-positive immunostaining (Fig. 2D) even in satellite neoplastic cell foci that had infiltrated the *pars distalis*. Markers for prolactin (PRL), glial fibrillary acidic protein (GFAP), and neuron-specific enolase (NSE) in the pituitary were negative (Table 1).

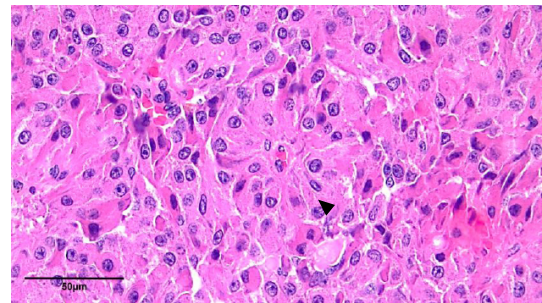
**Table 1.** Primary antibodies used for immunohistochemical study of *pars intermedia* neoplasia.

Antibodies	Clon	Dilution	Species produced	Antigen retrieval method	Detection kit
ACTH	O2A3	1:800	Mouse	HIER*	HRP conjugated polymer
GFAP	GA5	1:2000	Mouse	HIER	HRP conjugated polymer
NSE	MRQ-55	1:120	Mouse	HIER	HRP conjugated polymer
PROLACTIN	EP193	RTU**	Mouse	HIER	HRP conjugated polymer

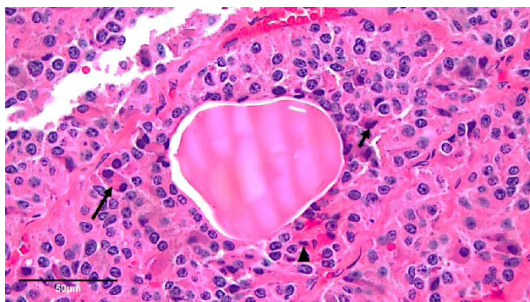
\*HIER: Heat-induced epitope retrieval; \*\*RTU; ready to use.



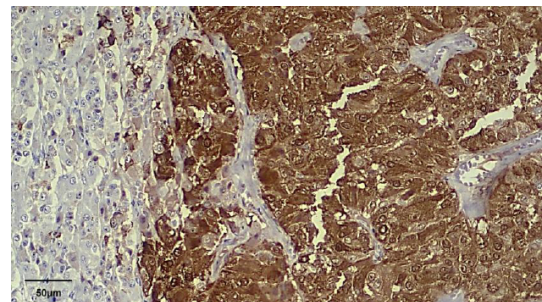
**Figure 2A**



**Figure 2B**



**Figure 2C**



**Figure 2D**

**Figure 2.** **A.** *Pars intermedia* adenoma of the pituitary gland in a horse. Two adjacent nodules of proliferating neoplastic cells of the *pars intermedia* were observed. The neoplastic cells were organized in cords or nests delimited by fine connective tissue and capillaries. The neoplastic nodules (asterisk) protrude, compress, and displace the neurohypophysis located on the right side. H&E. x100. **B.** Palisade cells were observed around blood vessels forming pseudorosettes (arrowhead). H&E. x400. **C.** The growth pattern of the neoplastic cells was organized as thyroid follicle-like structures with accumulation of secreted proteinaceous colloid material. Binucleated cells (long arrow), amorphous nuclei (short arrow), and mitotic figure (arrowhead). H&E. x400. **D.** Intracytoplasmic and perinuclear immunostaining for ACTH of the neoplastic cells. IHC x200.

A nodule of the pituitary *pars intermedia* neoplasm was preserved in glutaraldehyde buffered in 2.5% PBS for 24 h. Samples were subsequently fixed in 1% osmium tetroxide (1 h) and processed for electron microscopy. Ultrastructural analysis revealed neoplastic cells with cytoplasmic granules of variable electron density and with diameters between 160-250 nm. Multiple mitochondria, smooth

endoplasmic reticulum, and well-developed rough endoplasmic reticulum were observed. The neoplastic cells showed round or oval nuclei with shallow indentations, and some had prominent nucleoli. Mild anisokaryosis, abundant euchromatin diffusely distributed in the nucleus, and heterochromatin attached to the nuclear membrane were noted (Fig. 3).

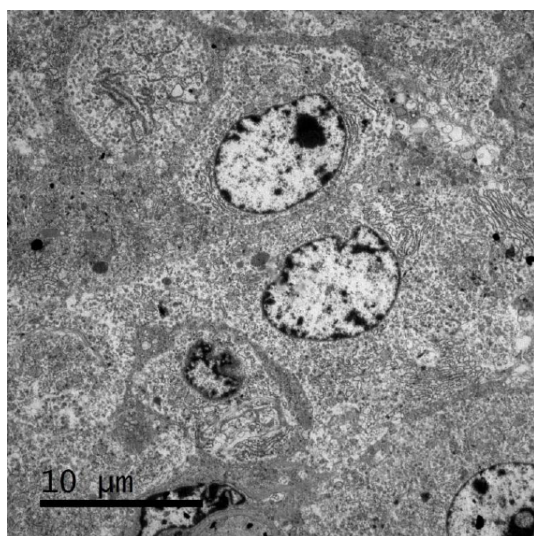


Figure 3A

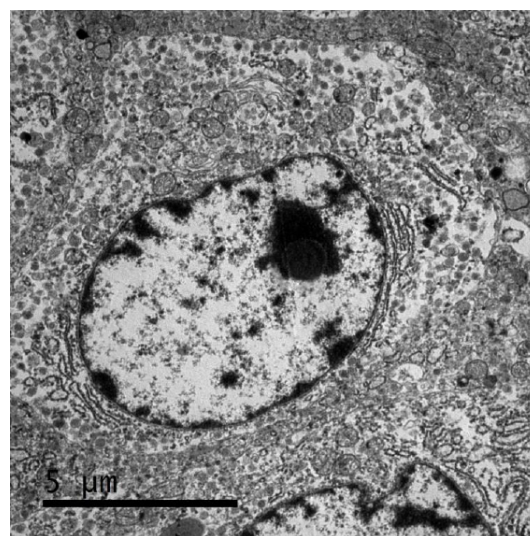


Figure 3B

**Figure 3.** Ultrastructure evaluation of the adenoma of the *pars intermedia*. **A.** Polyhedral, oval or cubic cells with irregular cytoplasmic borders; cleft nuclei; abundant, randomly distributed secretory granules of different electron densities in the cytoplasm. TEM. **B.** High magnification detailing the secretory granules, multiple mitochondria, and well-developed rough endoplasmic reticulum with abundant ribosomes. TEM.

## Discussion

An Argentine Saddle Horse presented clinical signs that included poor body condition, chronic body weakness and recurrent pathological decubitus. Necropsy revealed an enlarged pituitary gland, severe hirsutism, and severe diffuse muscle atrophy. Light microscopy, IHC and TEM confirmed the presence of morphological changes consistent with ACTH-producing PI adenoma of horses. The morbidity was 1.8% (1/57) and mortality was 1.8% (1/57). This neoplasm occurs sporadically. The clinical signs in the case presented here were similar to those reported in other horses with ACTH-

producing PI adenomas (Boujon et al., 1993; Yoshikawa et al., 2001; Gris et al., 2023).

The pituitary gland protruded from the sella turcica and the height of the gland was 2.8 cm. This macroscopic finding suggested an adenoma of the pituitary gland. Miller et al. (2008) described adenomas of the pituitary gland in four horses, in which the average height of the pituitary glands was greater than 1.6 cm. According to the criteria for histological classification of pituitary gland lesions in horses, which include grades from 1 to 5, pituitary lesions with a diameter > 0.5 cm are classified as grade 5 and correspond to adenomas (Miller



et al., 2008). Thus, the histological lesion in the pituitary gland of this case was classified as a grade 5 adenoma.

The morphological patterns of the neoplasia described here were similar to those reported in other PI adenomas (Miller et al., 2008; Meuten, 2017). The cellular morphology of neoplastic cells and the highly homogeneous nuclear size correspond to those described by others (Boujon et al., 1993; Yoshikawa et al., 2001; Meuten, 2017). In addition, at least four mitotic figures were counted in 2.37mm<sup>2</sup>. Some binucleated cells, mild megalocytosis, mild megalokaryosis and a few aberrant nuclei were also found.

Morphological findings in the skin are consistent with hormonal dermatosis (Meuten, 2017) and have been described in horses with PPID (Spelta, 2015; Gris et al., 2023). However, the mechanism behind these changes is unknown; it has been suggested that overproduction of POMC and a consequent increase in ACTH influence follicular development in the anagen phase has been suggested (Morgan et al., 2018). Strong immunodetection of the ACTH hormone in neoplastic cells of the PI adenoma explains the skin lesions. However, this should be confirmed by serum ACTH levels.

Histologically, necrotic, neutrophilic, fibrinous enterocolitis with intralesional bacteria was observed. This finding represents an acute infection. Additionally, pyogranulomatous hepatitis was found. Both events might be explained by subclinical bacterial and/or parasitic infections although microbiology and parasitology were not conducted in this study. McFarlane et al. (2015) described frequent secondary infections associated with PI adenomas and hypothesized that they result from loss of regulation of the immune system or the influence of increased ACTH levels. We propose that the animal was immunosuppressed due to neoplasia.

The ultrastructural findings of the neoplastic cells were similar to those described for tumors with secretory activity (Horvath and Kovacs,

1976). In the case presented here, granules measuring between 160-250 nm in diameter were found, and they also appeared in different stages of secretion, which was similar to those described for ACTH containing secretory granules (Boujon et al., 1993; Yoshikawa et al., 2001; Osamura et al., 2008).

The rough endoplasmic reticulum and Golgi apparatus were well developed with abundant mitochondria; such findings were similar to those described in ACTH-producing PI adenomas (Boujon et al., 1993). However, TEM revealed multifocal secretory activity, which might alter the visualization of granules and their electron density (Mete et al., 2016). Caution is recommended when analyzing neoplasms of the pituitary gland in horses using TEM alone, and diagnosis should be based on findings obtained with other diagnostic tests such as IHC (Yoshikawa et al., 2001; Mete et al., 2016). In this case, the ultrastructural findings obtained by TEM were complemented by positive intracytoplasmic immunostaining for ACTH using IHC, supporting the diagnosis of a PI adenoma in a horse.

Pituitary PI neoplasia has been described in several horse breeds (Boujon et al., 1993; Yoshikawa et al., 2001; Miller et al., 2016; Gris et al., 2023). However, to the authors' knowledge, this neoplasia has not been described in Argentine Saddle Horses and horses raised in Colombia. For this reason, this study constitutes the first report of pituitary PI neoplasia in this horse breed and contributes to the available information on the disease in horses. There are few reports of pituitary gland neoplasia that include a comprehensive morphological study and confirmation using IHC and TEM in the literature.

In conclusion, this is the first comprehensive study describing a pituitary PI adenoma using electron and light microscopy along with immunohistochemistry in a horse in Colombia and South America, and the first in the Argentine Saddle Horse breed.

## Declarations

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## Conflicts of interest

The authors declare they have no conflicts of interest regarding the work presented in this report.

## Author contributions

Necropsy, histopathology, literature review and writing of the first manuscript: DGG and BDD. IHC, TEM and critical revision of the manuscript: PCEC and BDD. All authors reviewed and approved the final version.

## Use of AI

No AI or AI-assisted technologies were used during the preparation of this work.

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