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Holoprosencephaly associated with synophthalmia and arrhinia in feline (*Felis catus*): a case report

*Holoprosencefalia associada a sinoftalmia e arinia em felino (*Felis catus*): relato de caso*

Juliana Sousa Terada Nascimento[1]*, Átila Bezerra de Mira[1], Kelly Cristina de Araújo Barbosa[1], Sara Preato de Oliveira[1], Maria de Lurdes Silva Ardição[1], Sandro de Vargas Schons[1]

ABSTRACT: The aim of this study was to describe the macroscopic and histological lesions observed in domestic newborn felines with facial lesions characterized by holoprosencephaly associated with synophthalmia and arhinia. A feline with multiple craniofacial congenital anomalies was necropsied, and the brain was collected, followed by photodocumentation and histopathological processing with H&E. The diagnosis of alobar holoprosencephaly with grade (I) morphological lesions of the face was made through the anatomopathological changes observed during the clinical and necroscopic examination, characterized by deformation of the cerebral hemispheres, shown failure in the forebrain vesicle development, located in the cranial neural tube part, associated with craniofacial dysmorphisms. In the microscopic study of the CNS, lesions characterized by abnormal lamination of the cerebral hemisphere's outermost cell layers and multiple areas of vacuolization of the cortex and in the cerebellum low cell density of the granular layer, with the presence of large intracytoplasmic vacuoles and absence of Purkinje cells were observed. It was not possible to determine the deformation cause, since laboratory tests were not performed, as well as the association with the FeLV virus.

KEYWORDS: Anomalies; Cat; Cyclopia; Holoprosencephaly.

RESUMO: O objetivo deste estudo foi descrever as lesões macroscópicas e histológicas observadas em felino doméstico neonato com lesões de face caraterizadas por holoprosencefalia associada à sinoftalmia e arinia. Um felino com múltiplas anomalias congênitas craniofaciais foi necropsiado, sendo coletado o encéfalo, em seguida foi conduzida a fotodocumentação e processamento histopatológico com H&E. O diagnóstico de holoprosencefalia alobar com lesões morfológicas da face de grau (I)' foram realizadas por meio das alterações anatomopatológicas observadas durante o exame clínico e necroscópico, caracterizadas pela malformação dos hemisférios cerebrais, evidenciando falha no desenvolvimento da vesícula prosencefálica, localizada na parte cranial do tubo neural, associado as dismorfias craniofaciais. No estudo microscópio do SNC foram observadas lesões caracterizadas por laminação anormal das camadas celulares mais externas dos hemisférios cerebrais e múltiplas áreas de vacuolização do córtex e no cerebelo pouca densidade celular da camada granular, com presença de grandes vacúolos intracitoplasmáticos e ausência das células de Purkinje. Não foi possível determinar a causa da má formação, uma vez que não foram realizados testes laboratoriais, bem como a associação com o vírus FeLV.

PALAVRAS-CHAVE: Anomalias; Ciclopia; Gato; Holoprosencefalia.

INTRODUCTION

Congenital anomalies are morphological and functional deformations of tissues, organs, and/or systems, caused by genetic and/or environmental factors, such as infectious agents, toxic, chemical, nutritional agents, and physical aggressions. In animals, such anomalies are poorly reported and the etiology is usually uncertain (Winter; Kennedy; Woodwaard, 2015). In part, it is due to low notification, especially observed in developing countries (Norman *et al.*, 1995; Southard *et al.*, 2016).

Among the teratogenic anomalies, those related to CNS deformation are the most disabling, being the severity of the lesions positively correlated with the extent of brain deformity, among these, are: anencephaly, amyelia, exencephaly, spina bifida, hydrocephaly, and holoprosencephaly (HPE) (Blecowe *et al.*, 2010). HPE, also known as "monster fetuses", results

[1] Fundação Universidade Federal de Rondônia, Departamento de Medicina Veterinária, Campus de Rolim de Moura/ Rolim de Moura/RO, Brasil
*Corresponding author: sandroschons@unir.br
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from a failure in the cleavage of the anterior brain, remaining small, undivided, and often merging the lateral ventricles, forming a single and large ventricle (Tekendo-Ngongang; Muenke; Kruszka, 2020). Defects in the embryogenesis of the anterior brain often result in craniofacial deformities, such as cyclopia, sinophthalmia, etmocephaly, cebocephaly, microphthalmia, arhinia and premaxillary agenesis (Glaze, 2005). Such morphological alterations of the face are associated with failure of the development of craniofacial structures, still in the embryonic formation stage (Yamada *et al.*, 2006).

In humans, HPE is generally related to specific or chromosomal mutations and phenotypic factors such as maternal health status, nutritional deficiencies, and exposure to teratogenic agents (Hamali; Nader, 2010). Studies attribute the deformations and mutations to the " Sonic hedgehog" (SHH) signaling peptides, which are expressed in the notochord, neural tube, limbs, and intestine, necessary for the normal development of the central nervous system (Cohen JR, 2010). However, due to the complexity of the factors involved, in only 15 to 20% of the cases, it is possible to determine the cause, because it is usually due to a set of events of polygenic errors and synergistic interactions of teratogens (Brent, 2004; Orioli; Castilla, 2010).

There are no studies of HPE prevalence in animals, however, in humans, 1 (one) is expected every 15,000 to 20,000 live births (Moore; Persaud; Torchia, 2016). At the same time, defects reported in humans with HPE resemble cases described in animals (Gongal; Wakiewicz, 2008).

Based on the above, the aim of this study was to describe the macroscopic and histological lesions observed in a case of holoprosencephaly associated with synophthalmia and arhinia in neonatal domestic felines.

CASE REPORT

A cat, without a defined breed, female, with multiple craniofacial congenital anomalies, died two days after birth. According to the tutor, no clinical changes were observed during the gestation period, and no treatment was performed. Of the six offspring, only the evaluated pup displayed congenital craniofacial lesions.

Soon after the death, a necroscopic examination was performed and the brain was collected for microscopic study. The samples collected were fixed in 10% buffered formalin for 96 hours. Subsequently, it dehydrated in a series of ethyl alcohol in increasing concentrations (70 to 100%) and diaphanized in xylene, with subsequent inclusion in histological liquid paraffin, microtomized in 4µm of thickness and stained with Hematoxylin-eosin (H&E).

RESULTS AND DISCUSSIONS

The diagnosis of holoprosencephaly was made through the anatomopathological alterations observed during the clinical and necroscopic examination, characterized by deformation of the cerebral hemispheres, shown a failure in the development of the prosencephalic vesicle, located in the cranial part of the neural tube, associated with craniofacial dysmorphism, similar to those described by Hajduk *et al.* (2011). HPE results from a failure in the cleavage of the forebrain (Moore, 2008). In its classical form, deformation is associated with facial alterations, such as hypotelorism with proboscis or single nasal orbit, cyclopia, labiopalatine cleft, and single central incisor (Calil; Krebs, 2011).

During the physical examination of the feline, alterations were observed characterized by the absence of ethmoid bone, turbinates, frontal sinuses, palatine, recess of the jaw, and external structures of the nostrils, with the union of the two eyeballs (Figure 1A). According to the classification of facial morphological alterations in proposed humans by Demyer and Zemam (1964), the fusion of orbits or the presence of two eyeballs very close to each other are classified as grade I anomalies (Cyclopia), and the absence of grade II nose (etmocephaly). Cyclopia is the most severe anomaly observed in cases of HPE in humans, and also the most frequent in necropsied fetuses, on the contrary, it occurs in cases of etmocephaly and is little reported (Noronha *et a*l., 2001). In animals, cyclopia has already been described in cattle (Pacheco *et al.*, 2011), pigs (Wammes *et al.*, 2012), and goats (Mota *et al.*, 2021). In a quarter horse fetus, oculars were present although no ocular structures or optic nerve were observed (Henker *et al.*, 2022).

In the necroscopic examination of the CNS, the brain had a brownish color, globose, smooth aspect, and soft consistency (Figure 1B), with the presence of an extracerebral, yellowish-brown cyst in the frontal cortex that ruptured during the removal of the brain, making it impossible to perform a microtomy of the region to study the tissue morphology (Figure 1B arrow). The presence of cysts covered by meningeal tissue located in the forebrain has also been described in horses (Henker *et al.*, 2022). In the brain, a fusion of the rostral components, the rostral longitudinal commissure,

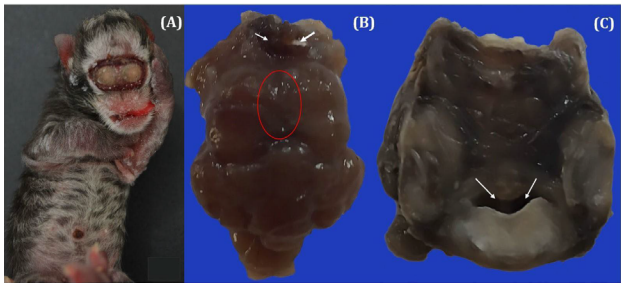

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Figure 1. (A) Feline with agenesis of the frontal bone, nasal absence, and a mid-side portion of the lacrimal bone. (B) Photomacrograph of the brain shows a globose, smooth appearance with the absence of the longitudinal fissure (red circle) and the union of the cerebral hemispheres. Presence of a ruptured cystic cavity during removal of the dura mater (arrow). (C) Photomacrograph of the ventral brain portion with the presence of a cavity of the remaining fourth ventricle (arrow).

aberrant circumvolutions (Figure 1B), and absence of olfactory bulbs were also observed, and in the ventral portion, the opening of a cavity possibly reminiscent of the fourth ventricle (Figure 1C arrows). According to Demyer (1971), brain lesions in human fetuses with HPE can be classified as, Alobar, Semilobar, Lobar, and Arrinencephaly.

In the alobar form, the cerebral hemispheres are not formed and have a supratentorial mono-ventricular cavity. In the semi-lobar type, partial cerebral hemisphere formation occurs, especially in the posterior portions, with the occipital and temporal horns identifiable and the frontal horns fused. In the lobar form, the cerebral hemispheres and ventricular cavities are normal, however, the fusion of the frontal horns persists, with the absence of lucid septum and hypoplasia of the frontal lobes (Demyer, 1971; Barr *et al.*, 1983; Leibovitz; Lerman-Sagie; Haddad, 2022), and in arrinencephaly morphological changes are observed characterized by the isolated absence of bulbs and olfactory tracts (Demyer, 1971; Leibovitz; Lerman-Sagie; Haddad, 2022). There is no classification of morphological lesions of the brain and face in animals, however, the defects reported in humans with HPE resemble those described in animals (Gongal; Wakiewicz, 2008). If we consider the classification proposed by Demyer and Zeman (1971) in humans, the lesions observed in the feline are classified as alobar HPE, with morphological lesions of the face of grade I.

The degree of brain development in classical holoprosencephaly is directly related to the severity of mesenchyme deficiency, so in alobar form, the cerebral hemispheres are not formed (Lamego; Barbosa-Coutinho, 1994). This explains the absence of longitudinal cerebral fissures, the union of the cerebral hemispheres and the single ventricle, similar to that observed in the feline brain and also in cases of HPE in humans (Norman *et al.*, 1995).

The absence of the olfactory bulb is often associated with human HPE (Noronha *et al.*, 2001; Barkovich *et al.*, 2005; Southard *et al.*, 2016), however, in the feline, the optic nerves were absent and the site was occupied by an extracerebral cyst, formed by ependymal glial tissue. Extracerebral cysts are often reported in cases of human lobar HPE (Rossing; Friede, 1992), however, it had not yet been reported in felines.

In the microscopic study of the nervous tissue, lesions were observed characterized by thickening of the cortex, abnormal laminating of the outermost cellular layers (Figure 2A), with multiple vacuolation areas in several regions of the cortex, ranging from discrete to accentuated (Figures 2B and 2C (arrow). Excessive thickening of the cortex portion and abnormal laminating of the cell layers are described in cases of human HPE and are indicative of disturbance of neuronal migration during embryogenesis (Mizuguchi; Morimatsu, 1989). However, such injuries had not been described yet in cases of felines with HPE.

In the telencephalon, there was a reduction in the number of cells in the molecular and external granular layers, resulting in little differentiation between gray and white substances. As well as multiple areas of vacuolization and discrete gliosis with increased perivascular spaces. Similar histological changes were also observed in cattle with HHP (Dimuro *et al.*, 2020). Such cellular abnormalities in these regions of the cortex are due to the impairment of neuronal migration at the level of the granular layer (Mizuguchi; Morimatsu, 1989). In addition to the lesions described above, cavitations delimited by astrocytes were also observed, characterizing the formation of a glial limiting membrane of various sizes and filled by cerebrospinal fluid in the molecular layer of the occipital lobe.

The cerebellum showed low cell density of the granular layer, with the presence of large intracytoplasmic vacuoles and the absence of Purkinje cells (Figure 3B). Leptomeninges were distended by liquid, forming large cavities (Figure 3 A). Reduced cerebral parenchyma, with a thin layer of gray and white substance, with multiple areas of hemorrhages and hyperemic blood vessels were also observed in bovine with HPE (Dimuro *et al.*, 2020).

In veterinary medicine, HPE has been commonly reported in cattle poisoning by plants containing cyclopamine, such as *Veratrum californicu* (Pacheco *et al.*, 2011). Cyclopamine is an antagonist phytochemical of the natural pathway of SHH leading to deformations in the face and CNS during

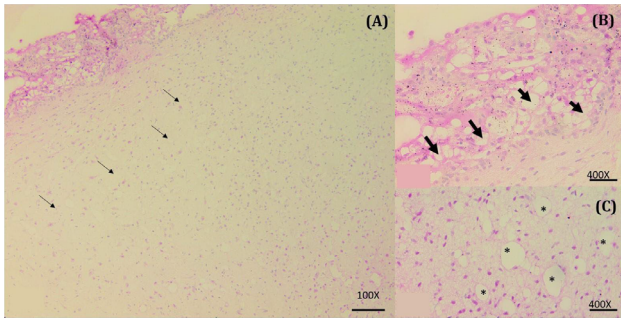

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Figure 2. (A) Photomicrographs of the frontal cortex with abnormal lamination of the outermost cell layers (arrow). (B) cellular vacuolation of the molecular layer (arrow). (C) vacuolation of nervous tissue (asterisk).

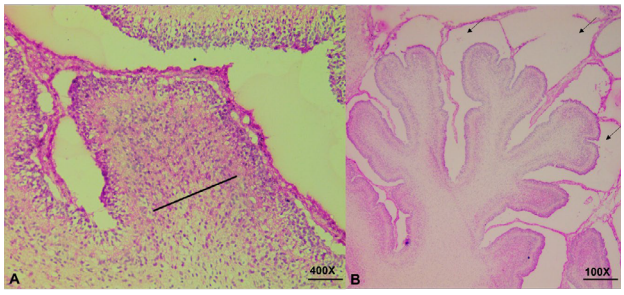

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Figure 3. (A) Photomicrographs of the cerebellum with reduced cell density in the granular layer and absence of Purkinje cells (Bar). (B) Presence of multiple cavities containing fluid in the pia mater (arrow).

embryogenesis (Hamali; Nader, 2010; Pacheco *et al.*, 2011). In addition to cattle, poisoning by *V. californicu* was also reported in horses (Koch *et al.*, 2005) and poisoned rabbits (Booth *et al.*, 2013). However, in carnivores, HPE is considered rare and is reported in Rottweiler (Martínez *et al.*, 2006) and Schnauzer (Miyama *et al.*, 2009) dogs.

Other teratogenic agents that directly or indirectly disrupt are associated with the occurrence of PEH in human and animal models. These include Retinoic acid, foodborne mycotoxins (such as ochratoxins), cyclopamine (an inhibitor of SHH signaling), and holes that interfere with cholesterol biosynthesis (Edison; Muenke *et al.*, 2003; Van Gelder *et al.*, 2010)

In cats, Feline leukemia virus (FELV) infection has been associated with cases of HPE, however, the pathogenesis related to changes during fetal development is not well elucidated yet (Southard *et al.*, 2016). In addition, toxic, stressor agents and antibiotic drugs in pregnant cats are considered possible factors that trigger brain deformations in feline neonates (Narfström, 1999; Slatter, 2005). Feline parvovirus showes an important teratogenic viral agent in cats and is related to the development of cerebellar hypoplasia in cats. Thus, the effects of teratogenic agents depend on the stage of gestation and the agent introduced (Southard *et al.*, 2016). However, in the current study, it was not possible to determine the cause of deformation, since laboratory tests were not performed to confirm the positivity of viruses such as FeLV.

CONCLUSIONS

Alobar Holoprosencephaly may be associated with other congenital alterations such as arhinia and synophthalmia that characterize their severity and correspond to changes incompatible with life. This dysmorphia is considered rare in cats; however, studies of its macroscopic and histopathological characteristics are important for comparative studies with the human species. It is concluded that there is a need for more in-depth studies about these deformations so that there is a better understanding of their causes.

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