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Topic of General Interest
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Straightforward identification of structures of the striatum in bovine and equine forebrains: Guidance to trimming[1]

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ABSTRACT.- Gomes A.A., Feitosa G.A., Pisco R.M., Tondo L.A.S., Lopes P.R.B., Abidu-Figueiredo M., Ubiali D.G. & Alonso L.S. 2024. **Straightforward identification of structures of the striatum in bovine and equine forebrains: Guidance to trimming.** *Pesquisa Veterinária Brasileira 44:e07423, 2024.* Laboratório de Ensino, Pesquisa e Extensão em Anatomia Comparada dos Vertebrados, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, Rodovia BR-465 Km 7, Seropédica, RJ 23890-000, Brazil. E-mail: danielubiali@ufrrj.br

Standardizing trimming the brain for histological diagnosis of neurological diseases is challenging for veterinary pathologists. The striatum is a set of subcortical nuclei of the forebrain, formed by bundles of nerve fibers and gray matter, which consist of the internal and external capsules, caudate, lentiform, and claustrum nuclei. The striatum is related to motricity, which refers to the neural circuits of the extrapyramidal pathways. In domestic animals, the extrapyramidal pathways are of great importance in gait. From the ventral surface of the brain, they are located deep in a strip of the telencephalon corresponding to the olfactory trigone. Bovine and equine brains were routinely formalin-fixed, weighed and measured. Herein, we report a guide for bovine and equine brains' neuroanatomic trimming and striatum histomorphology. Trimming was performed, and the sections were routinely processed for histology. We used the cross-section rostral to the optic chiasm as the site of choice for histologic sampling to identify the striatum structures. The forebrains were sectioned, and the standard histologic cassette was positioned horizontally or vertically to frame a greater diversity of structures. The histologic slides were labeled with structures of the striatum (caudate nucleus, internal capsule, lentiform nucleus, external capsule and claustrum). Regarding the brain size, handing the cassette orientation over to the forebrain allows the verification to shift the position or collect a second striatum sample for histology.

INDEX TERMS: Neuroanatomy, neuropathology, central nervous system, histopathology, trimming.

RESUMO.- [**Identificação direta de estruturas do corpo estriado em cérebros bovinos e equinos: uma orientação para clivagem.**] Padronizar a clivagem do encéfalo para diagnóstico histológico de doenças neurológicas é um desafio para os patologistas veterinários. O corpo estriado é um conjunto de núcleos subcorticais do prosencéfalo, formado por feixes de fibras nervosas e substância cinzenta, que consistem nas cápsulas interna e externa, núcleos caudado, lentiforme e claustro. O corpo estriado está relacionado à motricidade, que se refere aos circuitos neurais das vias extrapiramidais. Nos animais domésticos, as vias extrapiramidais são de grande importância na marcha. Da superfície ventral do cérebro, eles estão localizados profundamente em uma faixa do telencéfalo correspondente ao trígono olfatório. Encéfalos de bovinos e equinos foram rotineiramente fixados em formalina, pesados e medidos. Neste trabalho, relatamos um guia para a clivagem neuroanatômica e histomorfológica do corpo estriado dos cérebros de bovinos e equinos. A clivagem foi realizada e as seções foram processadas rotineiramente para histologia. Utilizamos o corte rostral e tangente ao quiasma óptico como local de escolha para amostragem

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histológica para identificar as estruturas do corpo estriado. Os prosencéfalos foram seccionados e o cassete histológico padrão foi posicionado horizontal ou verticalmente para enquadrar uma maior diversidade de estruturas. As estruturas do corpo estriado foram identificadas nas lâminas histológicas (núcleo caudado, cápsula interna, núcleo lentiforme, cápsula externa e claustro). Conforme o tamanho do encéfalo, o manuseio do cassete sobre o prosencéfalo permite orientar a necessidade de alterar a posição ou a coleta de uma segunda amostra do corpo estriado para histologia.

TERMOS DE INDEXAÇÃO: Neuroanatomia, neuropatologia, sistema nervoso central, histopatologia, clivagem.

INTRODUCTION

The standardization of brain sample sections and histological processing techniques can be widely improved in multiple animal species to contribute to neuropathology (Summers et al. 1995, Barros & Marques 2003). Horses and cattle can be models for understanding nervous system diseases, and efforts to standardize neurohistopathology have already been considered (Bitschi et al. 2020). In domestic animals, the extrapyramidal motor circuit generally involves the telencephalon's striatum and the midbrain's red nucleus, which is essential in locomotion (Lahunta & Glass 2009).

The diseases affecting Brazil's cattle nervous system are highlighted due to high frequency and mortality rates (Barros et al. 2006). The main neurological diseases that affect cattle include rabies, meningoencephalitis by bovine herpesvirus, malignant catarrhal fever, botulism, suppurative meningitis, encephalic abscesses, listeriosis, thrombotic meningoencephalitis, and polioencephalomalacia (Sanches et al. 2000, Galiza et al. 2010, Ribas et al. 2013, Rondelli et al. 2017, Terra et al. 2018, Barbosa et al. 2022, Bromberger et al. 2023). Rabies is one of the most common neurological diseases in cattle in Brazil; about 40 thousand cattle die per year, resulting in significant economic losses (Mello et al. 2019). Internationally, the importance of diagnosing neurological diseases in cattle has increased dramatically since the 1980s due to the emergence of bovine spongiform encephalopathy (BSE) (Wells et al. 1987). The importance of bovine disease surveillance rises due to a public health issue associated with the human prionic disease called variant of Creutzfeldt-Jakob with BSE in the United Kingdom (Almond & Pattison 1997). Further, international health authorities request that cattle-producing and exporting countries monitor BSE in bovine herds and identify several diseases affecting the nervous system.

The main neurological diseases of horses are hepatic encephalopathy, leukoencephalomalacia, equine protozoal myeloencephalitis, arboviral encephalomyelitis, and rabies (Pimentel et al. 2009, Rech & Barros 2015). Due to high prevalence, bovine and equine species are hotspot species in the "Programa Nacional de Controle da Raiva dos Herbívoros" (National Herbivore Rabies Control Program) (Brasil 2010). Herein, we describe the location of the subcortical forebrain, aiming to localize the structures of the striatum in bovine and equine brains.

MATERIALS AND METHODS

Ethical approval. Forty domestic herbivores were submitted to the *post mortem* examination after death unrelated to the nervous system. No animals were euthanized for this project. Ethical statement was not necessary in the given context of the research.

Twenty-nine brains routinely fixed in 20% formaldehyde from horses (N=29) and eleven from cattle (N=11), without lesions, were accessed at "Setor de Anatomia Patológica" (SAP) from "Universidade Federal Rural do Rio de Janeiro" (UFRuralRJ). The removal of the brains was routinely carried out using a hatchet, scissors and forceps, and the brains were fixed entirely for histological examination (Barros & Marques 2003). Weight and measurements of length and width of the brains were obtained with a precision scale and caliper, and the averages were calculated with the respective standard deviations at "Laboratório de Ensino, Pesquisa e Extensão em Anatomia Comparada dos Vertebrados" (DAAH/ICBS) from UFRuralRJ.

Trimming of the forebrain. Initially, the cerebellum and brainstem were separated from the forebrain by section at the midbrain level. The forebrains were transversely sectioned rostral tangent at the level of the optic chiasm (Fig.1), generating gross forebrain slides used for trimming. A standard histologic plastic cassette with the bottom grid removed was used to demonstrate the tissue frame to trimming.

Fig.1. Striatum guidance to trimming. Ventral view of the fixed brain of a cow, demonstrating the local to the cross-section rostral tangent to the optic chiasm.

Histological processing. After trimming, fragments were vertically or horizontally included in the histological cassette according to the dimensions of the sample. The tissues were routinely processed for histological examination and stained by hematoxylin and eosin (HE). The gross and submacroscopic slides were compared and photographed to describe the nuclei.

RESULTS AND DISCUSSION

The average weight of bovine brains was 400.0g ± 85.0g, and equine brains were 380.0g ± 69.0g. In cattle, the total length and width of the brain were 12.0cm ± 1.2cm and 9.1cm ± 0.64cm, respectively. In horses, the length was 12.0cm ± 0.93cm, and the width was 8.52 ± 0.73cm. Overall, in horses and cattle, 12.5% (5/40) were 13 to 24 months of age, 42,5% (17/40) were 25 to 60 months of age, and 45% (18/40) were more than 60 months of age.

At the transverse forebrain level, ventrally tangent to the optic chiasm (Fig.1), there was homogeneity in the anatomy of the nuclei from examined bovines and equines, observed framing using the cassette plastic contour adapted to demonstrate trimming (Fig.2-3). For the gross and histological images, the anatomical regions of the striatum were framed at the caudate nuclei, internal capsule, lentiform nucleus, claustrum, cingulate gyrus and hypothalamus. The vertical position could not frame the entire striatum, tangentially cross-sectioned rostrally to the optic chiasm in adult domestic herbivores (Fig.4). We recommend obtaining a slice rostrally to frame the striatum in a vertical position in larger brains. For adult cattle and horses (larger width and height forebrains), the striatum was framed entirely in the horizontal position of the cassette. In this view, it is possible to identify the lentiform nucleus comprising the putamen and the globus pallidus, which includes the lamina medullaris lateralis and the lamina

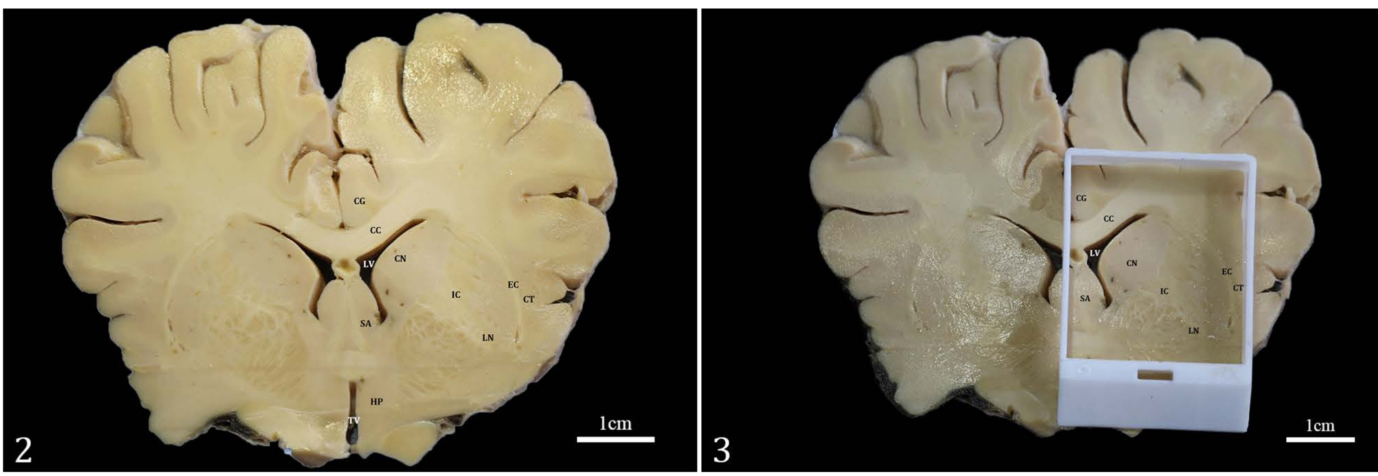

Fig.2-3. Striatum guidance to trimming. Fixed forebrain tangentially cross-sectioned rostrally to the optic chiasm of a bovine. (**2**) This view represents the cingulate gyrus (CG), corpus callosum (CC), lateral ventricle (LV), caudate nucleus (CN), internal capsule (IC), lentiform nucleus (LN), external capsule (EC), claustrum (CT), septal area (SA), hypothalamus (HP) and third ventricle (TV). (**3**) The same gross section demonstrating the histological cassette framing the striatum.

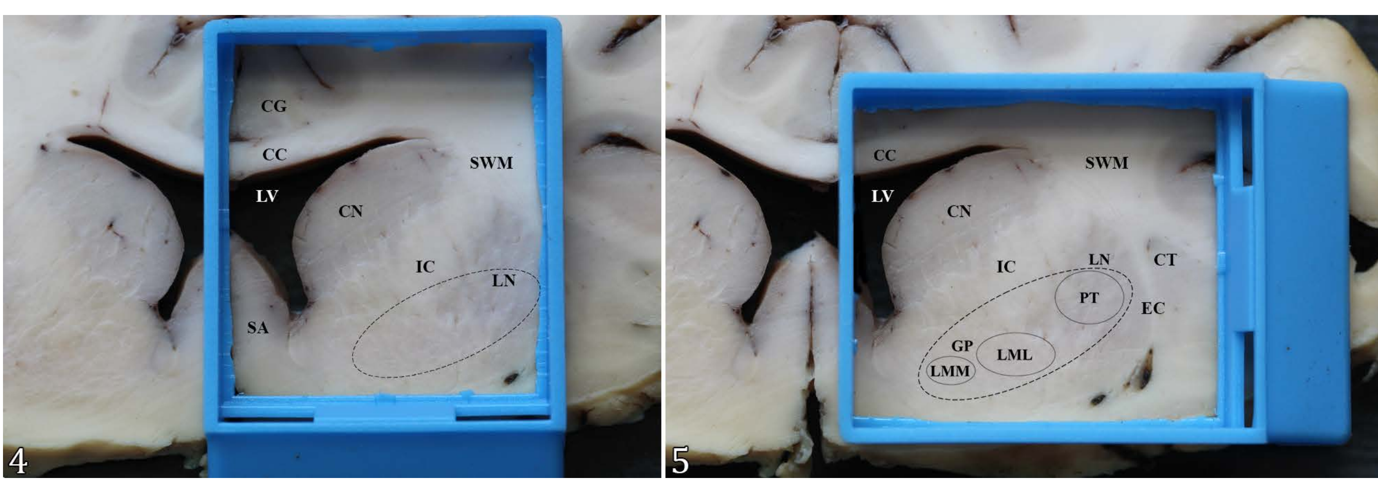

Fig.4-5. Striatum guidance to trimming. Fixed forebrain tangentially cross-sectioned rostrally to the optic chiasm of a horse. (**4**) Cross-section framing striatum structures in the histological cassette at vertical position demonstrating cingulate gyrus (CG), corpus callosum (CC), lateral ventricle (LV), septal area (SA), caudate nucleus (CN), internal capsule (IC), subcortical white matter (SWM) and lentiform nucleus (LN). (**5**) Cross-section framing striatum structures in the histological cassette at horizontal position demonstrating the subdivision of LN. The LN comprises the putamen (PT) and the globus pallidus (GP). The GP comprises the lamina medullaris medialis (LMM) and the lamina medullaris lateralis (LML). At the horizontal cassette position, it also frames external capsule (EC), claustrum (CT) and the SWM.

medullaris medialis. At the horizontal cassette position, it also frames external capsule (EC), claustrum (CT) and the SWM (Fig.5). Each slice of the corresponding striatum structures was identified inside the cassette (Fig.6). The professional must evaluate the indicated position to frame the striatum for trimming and further achieve the histological slide (Fig.7).

According to the Brazilian official guidelines, a cross-section of the forebrain at the level of the frontal cortex, rostral to the optic chiasm, represents an additional section for sampling the brain for the histological diagnosis of bovine spongiform encephalopathy (Barros & Marques 2003). The transverse sections are a traditional plane in the routine histopathology laboratory. Based on our experience, localizing striatum structures based on the dorsal gyri and sulci does not work due to individual anatomic variation. The ventral section of the forebrain, at the rostral level tangent to the optic chiasm, resulted in basal nuclei structures that did not vary in the individuals of this study, considering a site for trimming pattern.

This research approach presents a fast guide to identifying the caudate nucleus, internal capsule, lentiform nucleus, external capsule and claustrum, favoring accurate histological diagnoses. Basal ganglia are essential for domestic herbivores' motor functions and behavior expression (Lahunta & Glass 2009). Interneurons in the human striatum have relevant implications for the physiology and pathophysiology of neurological and psychiatric diseases. The striatum pathways are involved in complex neurological and psychiatric diseases affecting this set of brain structures (Garma et al. 2024). The human cortico-basal ganglia circuit is crucial for acquiring and executing motor skills, and neuron activities in these regions are linked to movement parameters and learning mechanisms. The synaptic pathways in the striatum are critical for motor skill acquisition, and disruptions in this plasticity can cause movement disorders (Roth & Ding 2024). The two most common human neurological disorders of the striatum are Parkinson's and Huntington's. These diseases' most evident clinical signs are motoric, reflecting the loss of specific neurons in the basal ganglia. Animal models of these diseases are useful for evaluating the viability of cognitive deficits and therapeutic development (Brooks & Dunnett 2013). The international task force for studying epilepsy in dogs and cats sought to standardize the processing of brains for histopathological examination. The purpose of standardization is to enhance the diagnosis of brain injuries by creating technical protocols that could be reproduced (Matiasek et al. 2015).

Considering the striatum as the set of nuclei at the base of the brain, we believe that the specific description of the location of lesions is adequate. We emphasize the importance of histomorphological and clinical correlations during the diagnosis exercise. Each institution will define its sampling regions for histomorphological studies or diagnostic purposes. The present guidance is a simple approach for defining striatum structures using globally standardized material for histological examination. However, there are current remarkable technologies for preparing entirely cross-sectional histological slides of large animal brains (Bitschi et al. 2020).

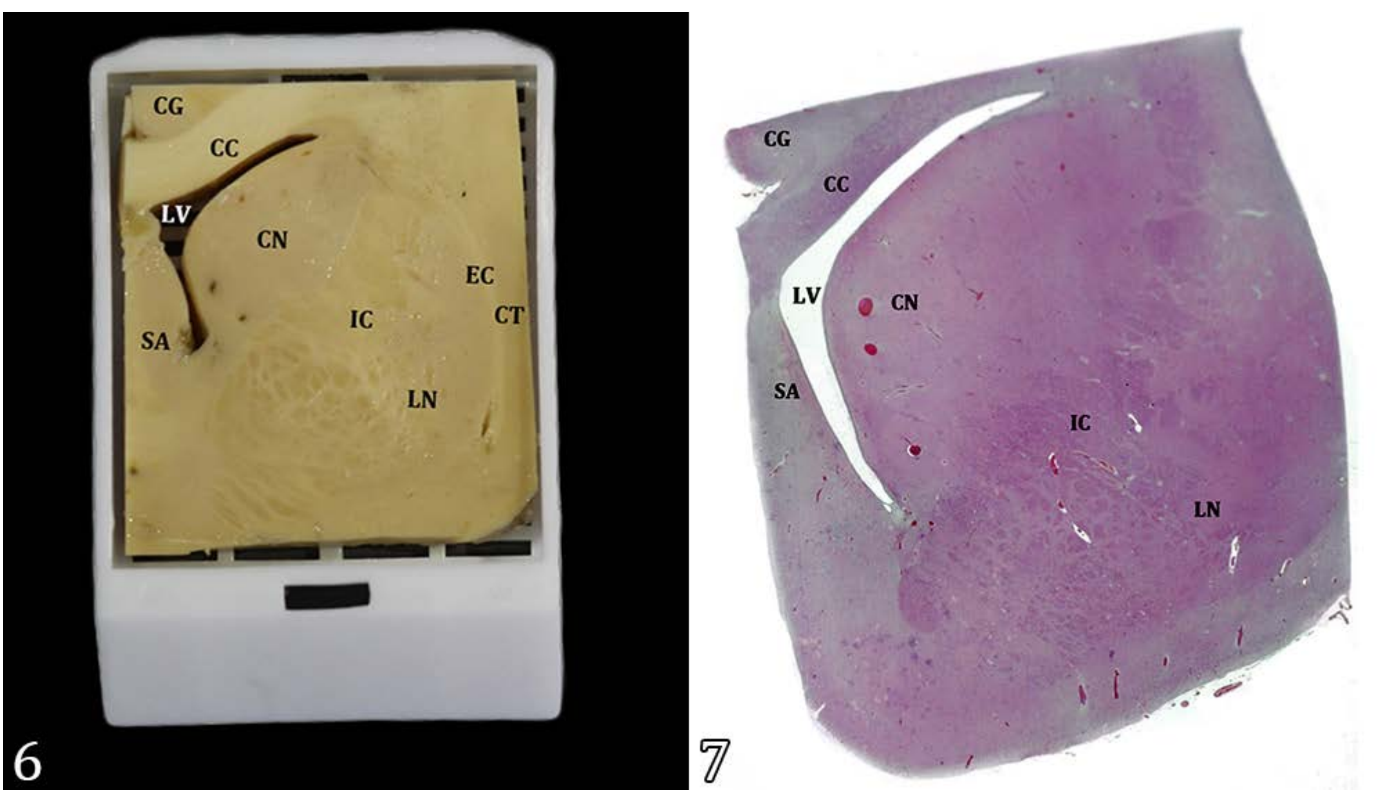

Fig.6-7. Striatum guidance to trimming. (**6**) Cross-section of fixed bovine forebrain framing striatum structures inside the histological cassette demonstrating cingulate gyrus (CG), corpus callosum (CC), lateral ventricle (LV), septal area (SA), caudate nucleus (CN), internal capsule (IC), lentiform nucleus (LN), external capsule (EC), claustrum (CT). (**7**) Histology of the striatum of an adult bovine showing CG, CC, LV, SA, CN, IC, and LN. Hematoxilin and eosin, submacroscopic view.

Of course, brain size has an effect on sampling for histology. As for the major brains, the diagnostician will select two tissue areas or hand the cassette over the tissue to verify the best position for greater coverage of the striatum areas. The vertical position was sufficient for young herbivores to frame the entire striatum. For adult horses and bovines, the wider distance from the midline to the claustrum should be reached in the horizontal position of the cassette. The vertical or horizontal position of the cassette should be a criterion of choice for the pathologist at the trimming time to fit the desired anatomical structures in the cassette.

CONCLUSIONS

The cross-section, rostral tangent to the optic chiasm, was the anatomic region to indicate the forebrain trimming at the striatum of cattle and horses.

Histological examination for diagnosing neurological syndromes that affect motor functions and behavior expression should investigate the striatum.

Authors' contributions.- Luciano Alonso and Daniel Ubiali: Conception. Aline Gomes, Guilherme Feitosa, Raquel Pisco, Paulo Lopes, Marcelo Abidu-Figueiredo and Luciano Alonso: neuroanatomic processing and evaluation. Daniel Ubiali, Luis Tondo and Luciano Alonso: neurohistological processing and evaluation.

Data availability.- The datasets used and analyzed during the current study are available from the corresponding author upon request.

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