PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research

ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 38(11):2099-2108, novembro 2018
DOI: 10.1590/1678-5150-PVB-6079

Topic of General Interest
Animais de Produção/*Livestock Diseases*

Gross and histopathological pitfalls found in the examination of 3,338 cattle brains submitted to the BSE surveillance program in Brazil[1]

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ABSTRACT.- Rech R.R., Giaretta P.R., Brown C. & Barros C.S.L. 2018. **Gross and histopathological pitfalls found in the examination of 3,338 cattle brains submitted to the BSE surveillance program in Brazil.** *Pesquisa Veterinária Brasileira 38(11):2099-2108*. Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Av. Senador Filinto Müller 2443, Campo Grande, MS 79074-460, Brazil. E-mail: claudioslbarros@uol.com.br

This study stems from the findings during the gross and histopathological exam of 3,338 cattle brains as part of the bovine spongiform encephalopathy (BSE) active surveillance program of the Brazilian Ministry of Agriculture, Livestock, and Supply from 2001 to 2005. The work was carried out in the Veterinary Pathology Laboratory of the Federal University of Santa Maria which at the time (2001-2007) was the national reference laboratory for the diagnosis of BSE and other transmissible spongiform encephalopathies. Both gross and histopathological aspects are described. Several gross aspects were annotated: anatomic normal structures not commonly recognized (non-lesions), lesions of no clinical significance, postmortem changes and artifacts; all these can amount to important pitfalls that distract the pathologist during the routine gross examination of the central nervous system (CNS). Accordingly, equivalent pitfalls were described in the histological examination. Non-lesions observed were the pineal body, embryo remnants such as the external germinal layer of the cerebellum, subependymal plates, and clusters of neuroblasts in the basal ganglia; or circumventricular structures such as area postrema, subcomisural organ, and melanosis in the leptomeninges and vessel walls. Lesions with little or no clinical importance included age-related changes as lipofuscin, hemosiderin, mineralization and hyalinization of vessel walls within the brain and meninges. *Corpora amylacea* and *corpora arenacea* were detected respectively in astrocyte processes and the pineal body. Cytoplasmic neuronal vacuolization was observed in the red nucleus and habenular nucleus. *Sarcocystis* sp. without a correspondent inflammatory reaction was rarely observed. Included within findings with no clinical manifestation were axonal spheroids and perivascular mononuclear cuffings. Changes in the CNS due to killing, sampling and fixation methods can obscure or distract from the more critical lesions. The ones related to the process of killing included hemorrhages caused in cattle destroyed by a captive bolt. Artifacts related to sampling and handling of CNS tissue consisted of inclusion of bone sand in the neural tissue from sawing the calvarium; dark neurons produced by excessive handling of the brain, and micro-organisms that contaminated the tissues during sampling or histological processing. Postmortem autolytic or putrefactive changes observed included vacuolar changes in the myelin sheath, clear halos surrounding neurons and oligodendrocytes, clusters of putrefaction bacilli within vessels or dispersed throughout the brain tissue associated or not to clear halos. One interesting, and somewhat frequent, postmortem autolytic change found in the bovine brain was the partial dissolution of the granule cell layer (GCL) of the cerebellum, also referred to as conglutination of the GCL

[1] Received on August 28, 2018.
Accepted for publication on September 6, 2018.
Part of the doctoral thesis of the first author.

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or as the French denomination "état glace". Due to the shortage of comprehensive publications in the subject, this review is intended to address the main pitfalls that can be observed in the brain of cattle hoping to help other pathologists avoiding misinterpret them.

INDEX TERMS: Postmortem findings, histopathology, pitfalls, cattle, brain, Brazil, central nervous system, artifacts, histology, neuropathology.

RESUMO.- [**Ciladas macroscópicas e histopatológicas encontradas no exame de 3.338 encéfalos de bovinos submetidos ao programa de vigilância da EEB no Brasil.**] Os resultados deste estudo foram obtidos pelo exame macroscópico e histopatológico de 3.338 cérebros de bovinos examinados durante o programa de vigilância ativa da encefalopatia espongiforme bovina (BSE) do Ministério da Agricultura, Pecuária e Abastecimento (MAPA), de 2001 a 2005. O trabalho foi realizado no Laboratório de Patologia Veterinária (LPV) da Universidade Federal de Santa Maria (UFSM) que, de 2001 a 2007, foi o laboratório nacional de referência para o diagnóstico da BSE e de outras encefalopatias espongiformes transmissíveis. Macroscopicamente, foram descritas estruturas anatômicas normais (não-lesões), mas que são, com frequência, interpretadas como lesões; lesões sem significado clínico; alterações pós-mortais e artefatos. Esses achados podem confundir e desviar a atenção do patologista durante o exame de rotina do sistema nervoso central (SNC). Da mesma forma, estruturas equivalentes foram descritas no exame histológico. As não-lesões observadas foram corpo pineal, remanescentes embrionários, como a camada germinativa externa do cerebelo, placas subependimárias e aglomerados de neuroblastos nos gânglios da base; ou estruturas circunventriculares, como área de postrema, órgão subcomissural e melanose em leptomeninges e paredes dos vasos. Lesões com pouca ou nenhuma importância relacionadas ao envelhecimento incluíram lipofuscina, hemossiderina, mineralização, hialinização das paredes dos vasos do encéfalo e das meninges. *Corpora amylacea* foram detectados em processos astrocíticos e *corpora arenacea*, no corpo pineal. Adicionalmente, foi observada vacuolização no citoplasma de neurônios do núcleo vermelho e do núcleo habenular. *Sarcocystis* sp. sem reação inflamatória correspondente foi raramente observado. Incluídos nos achados sem manifestação clínica estavam esferóides axonais e manguitos mononucleares perivasculares. Alterações no SNC causadas pelo método de abate, amostragem e fixação podem simular ou obscurecer lesões mais importantes. Aquelas relacionadas ao método de abate incluíram hemorragias causadas em bovinos dessensibilizados pelo dardo cativo ou por punção por faca da medula na articulação atlanto-occipital. Artefatos relacionados à amostragem e manuseio de tecido do SNC consistiram na inclusão de pó de osso no tecido neural em consequência do uso de serra para abrir a caixa craniana; neurônios escuros produzidos pelo manuseio excessivo do cérebro e micro-organismos que contaminaram os tecidos durante a amostragem ou processamento histológico. Alterações autolíticas pós-mortais ou de putrefação incluíram vacuolizações na bainha de mielina, halos claros em torno dos neurônios e oligodendrócitos, aglomerados de bacilos de putrefação dentro dos vasos ou dispersos em todo o tecido cerebral, relacionados ou não a halos claros. Uma alteração autolítica pós-mortal intrigante e relativamente frequente encontrada foi a dissolução parcial da camada de células granulares (CCG) do cerebelo, também referida como conglutinação da CCG ou "état glacé". Devido à escassez de publicações abrangentes neste assunto, esta revisão pretende abordar as principais ciladas que possam aparecer no cérebro de bovinos, na esperança de ajudar outros patologistas a evitar interpretá-las erroneamente.

TERMOS DE INDEXAÇÃO: Achados de necrópsia, histopatologia, encéfalo, bovinos, Brasil, sistema nervoso central, artefatos, histologia, neuropatologia.

INTRODUCTION

Identification of artifacts, normal structures that resemble lesions, lesions that are not associated with clinical signs, and postmortem changes, are an important aspect of diagnosing central nervous system (CNS) diseases, allowing for correct interpretation of clinical features of the disease. These changes are grouped in four main categories: non-lesions, lesions of no clinical significance, postmortem changes, and artifacts. In the non-lesions category are included morphologic aspects of the CNS examination that might be interpreted as lesions when the pathologist is not thoroughly familiar with the CNS. Lesions of no clinical significance include those that do not contribute to the altered clinical state of the animal and so are only incidental at necropsy or histologic examination. *Postmortem* changes are not real lesions, but occur after death as a result of autolysis or putrefaction. Artifacts can be produced due to killing, sampling and fixation methods.

During the high of the worldwide concern with BSE, the examination of a large number of bovine brains (Wells et al. 1991) became indispensable to achieve reliable surveillance of the disease. This study stems from the alterations described above during the gross and histopathological exam of 3,338 cattle brains as part of the bovine spongiform encephalopathy (BSE) active surveillance program of the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA) from 2001-2005. Due to the shortage of comprehensive publications in the subject this review is intended to address the main pitfalls that can be observed in the brain of cattle hoping to help other pathologists avoiding misinterpret them.

MACROSCOPIC EXAMINATION OF THE BRAIN

Non-lesions

In the adult cow and sheep, leptomeningeal melanosis is a frequent normal finding (Innes & Saunders 1962a). Leptomeningeal melanosis occurs as a non-lesion in cattle in Brazil, in breeds with pigmented skin such as Nelore and pigmented skin and dark hair coats such as Angus. Melanosis is characterized by black or dark brown areas in the leptomeninges, especially over the frontal lobes (Fig.1A) where it is frequently mistaken by cortical necrosis. This normal finding is also frequently seen in the brains of sheep from the breeds Suffolk and

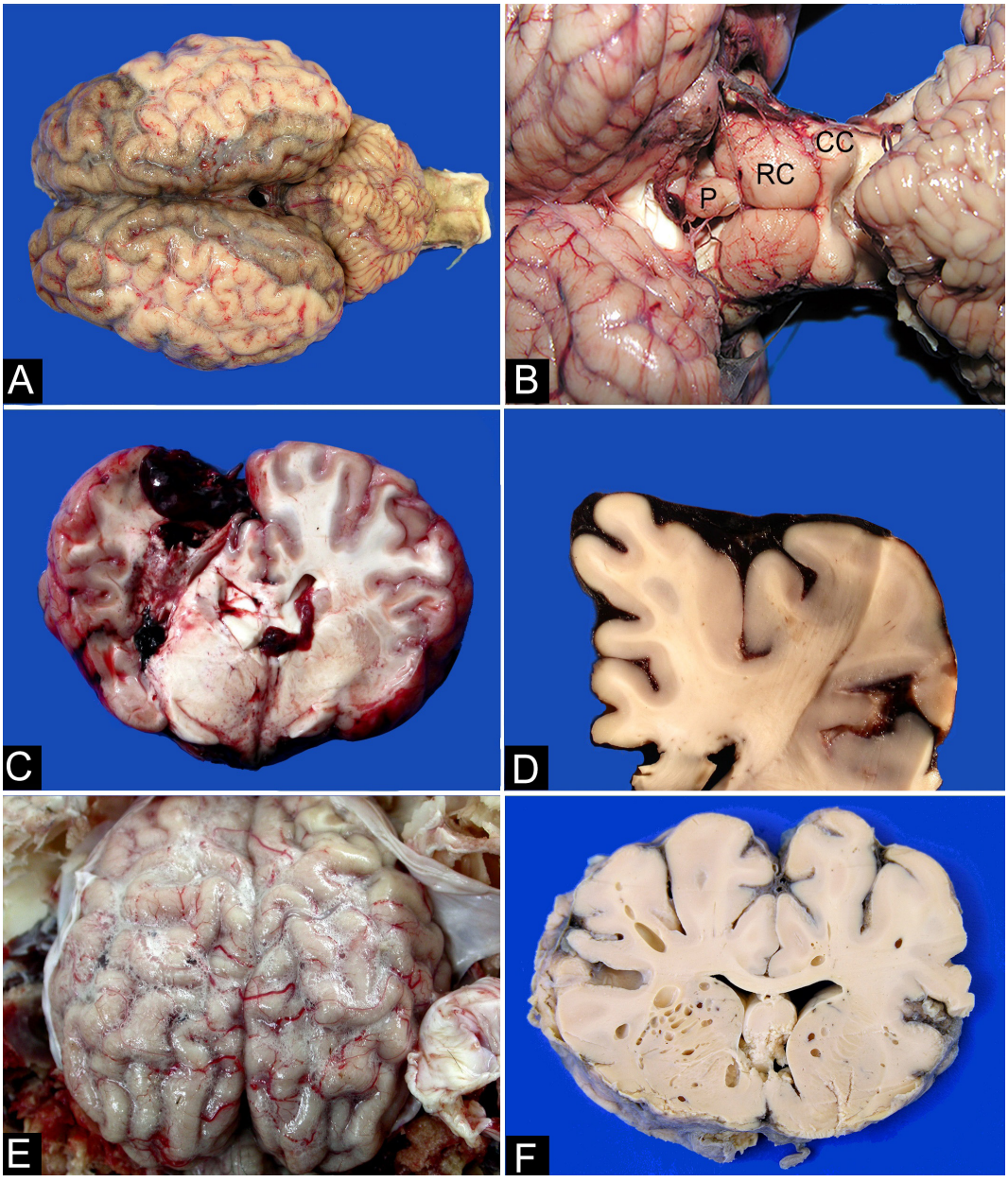

Fig.1. Macroscopic examination of the brain. (**A**) Frontal, parietal and occipital lobes of the brain with focally extensive leptomeningeal melanosis, a common normal finding in the bovine brain. See Figure 3F for histological appearance. (**B**) Location of the pineal gland (P) just rostral to the rostral colliculi (RC) of the midbrain and the thalamus. Caudal colliculi (CC). (**C**) Artifact due to the use of a captive bolt to insensibilize cattle before death by bleeding. Locally extensive area of subdural hemorrhage and a large fragment of bone is inserted in the brain parenchyma. (**D**) Artifact due to the use of a captive bolt to stun cattle before death by bleeding. Diffuse subdural hemorrhage is on the dorsal and lateral encephalic surfaces. (**E**) Artifact associated with the type of brain sampling. Air bubbles in the leptomeninges resulting from the ax's blows at the calvarium when removing the brain. (**F**) Postmortem putrefaction. Cross section of the brain at the level of the basal nuclei. Multiple variably-sized cavities in the brain parenchyma that resembles Swiss cheese.

Hampshire Down, where they can form impressive aggregates as to resemble a neoplasm (Summers et al. 1995). It occurs principally in the leptomeninges over the frontal lobes.

The *glandula pinealis* or pineal gland is a glandular structure (Gartner & Hiatt 2007) situated dorsally at the brainstem between the rostral colliculi of mesencephalic tectum and the thalamus (Fig.1B). It secretes melatonin and due to this glandular aspect that differs from the surrounding neural tissue, may be mistakenly interpreted as a neoplasm, especially on microscopic examination.

Artifacts due to the method of slaughter

The methods for killing and of removing the brain from the cranial cavity may create a variety of alterations in the bovine brain. Subdural hemorrhage is a lesion that can result from the method of euthanasia and is observed in brains from cattle that were killed by captive bolt (Fig.1C,D). A complete history in these cases is very important because it is difficult to differentiate these forms of hemorrhage from other causes of traumatic hemorrhage. Many times, when using a captive bolt for slaughter, fragments of bone can be found within brain

tissue. This can be attributed to the heavy pressure exerted by the captive bolt at the time of stunning (Coore et al. 2004). This heavy pressure can send emboli of brain tissue into the lungs and other organs such as liver (Garland et al. 1996). These emboli can assume several centimeters being promptly visible by the naked eye (Garland et al. 1996).

Artifacts due to sampling of the brain

The use of an axe for removal of the brain from the cranial vault produces bubbles in the leptomeninges. These bubbles are introduced in the leptomeningeal vessels while the prosector hits the bone with the axe (Barros et al. 2006). These bubbles are sometimes mistakenly interpreted as gas embolism (Fig.1E).

Postmortem changes

Postmortem changes include softening of the brain throughout, sometimes associated with the development of cavities caused by gases produced by the anaerobic bacilli of putrefaction. This may give the brain the appearance of a Swiss cheese (Fig.1F). It is important to distinguish Swiss cheese brain from necrosis or other true ante mortem lesion (Auer et al. 2008). The would-be neuropathologists may be trapped in diagnosing cystic necrosis when facing this type of postmortem change. However, the yellow color of necrosis is absent and, unlike in necrosis, the internal surface of the "holes" are smooth (Auer et al. 2008).

MICROSCOPIC EXAMINATION OF THE BRAIN

For optimal fixation for histopathologic examination, the bovine brain should be immersed in a volume of 10% neutral formalin fixative that is at least ten times greater than the volume of tissue to be fixed. Consequently, to adequately fix an entire bovine brain, 6 liters of 10% neutral formalin are needed. The time for fixation varies with the size of the brain. A brain from an adult bovine will require about 2 to 5 days before reaching full fixation. The sections, except the ones in Figure 2, are stained with hematoxylin-eosin (HE).

Microscopic examination of the brain should be carried out in a systematic way. To ensure representative analysis, diverse areas of brain are examined; it is recommended that both sides of serial transverse sections of 0.5 to 1cm be examined. The slides for histologic examination can be systematically processed from sections depicted in Figure 2. This procedure has the following advantages: permits detailed study of specific areas of the brain, offers consistent sections for comparison with control brains, and makes it easy to describe the distribution of lesions.

Non-lesions

Normal microscopic structures that might be mistaken for lesions include some remnants of embryonic structure (external layer of granule cells of the cerebellum, subependymal plaque), circumventricular organs or normal pigment in the vessels of the leptomeninges (melanin produced by melanocytes).

The external granule cell layer of the cerebellar cortex is normally found in fetuses, and calves up to a few months of age (Fig.3A). As the animal ages, these cells migrate toward the granule cell layer. In cattle, complete disappearance of this layer occurs between 12 and 18 months of age (Barros 1980). This external granule cell layer can be confused with

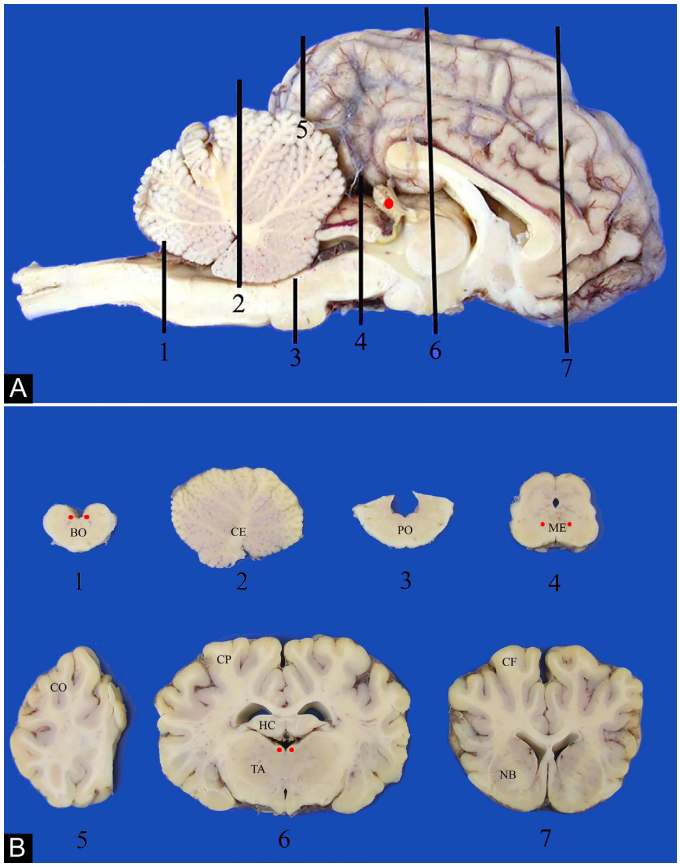

Fig.2. Bovine brain hemisphere showing a systematic way to obtain representative samples for histologic examination. (**A**) Medulla oblongata at the level of the obex, BO (1); Cerebellum, CE (2); Pons at the level of the cerebellar peduncles, PO (3); Midbrain at the level of the rostral colliculi, ME (4); Occipital lobe, CO (5); Diencephalon at the level of the thalamus: parietal lobe, CP, hippocampus, HC, and thalamus, TA (6); and frontal lobe, CF, at the level of the corpus callosum and basal nuclei, NB (7). Red dot = pineal gland. (**B**) Cross sections obtained from sampling of the brain in Figure 2A. Red dots = area postrema (section 1), red nuclei (section 4), habenular nuclei (section 6).

meningitis. In addition, these cells, as they migrate through the cerebellar cortex, can be confused with glial shruberries, a change seen when inflammatory cells accumulate in the molecular layer (Innes & Saunders 1962b).

Subependymal plaques can be localized in the lateral and ventral portions of the lateral ventricles and can be erroneously interpreted as ependymitis. Adjacent to the subependymal plaque and the olfactory bulb, neuroblast accumulations, known as residual glia, can be seen and these could be confused with areas of focal gliosis (Fig.3B).

Circumventricular organs are specialized centers that share two morphologic characteristics - periventricular location and vasculature lacking the typical blood-brain barrier organization (Duvernoy & Risold 2007, Vandevelde et al. 2012, Wohlsein et al. 2013). Circumventricular organs include: (a) subcommissural organ, a local modification of ependymal cells that projects into the mesencephalic aqueduct and which is located on the ventral surface of the posterior

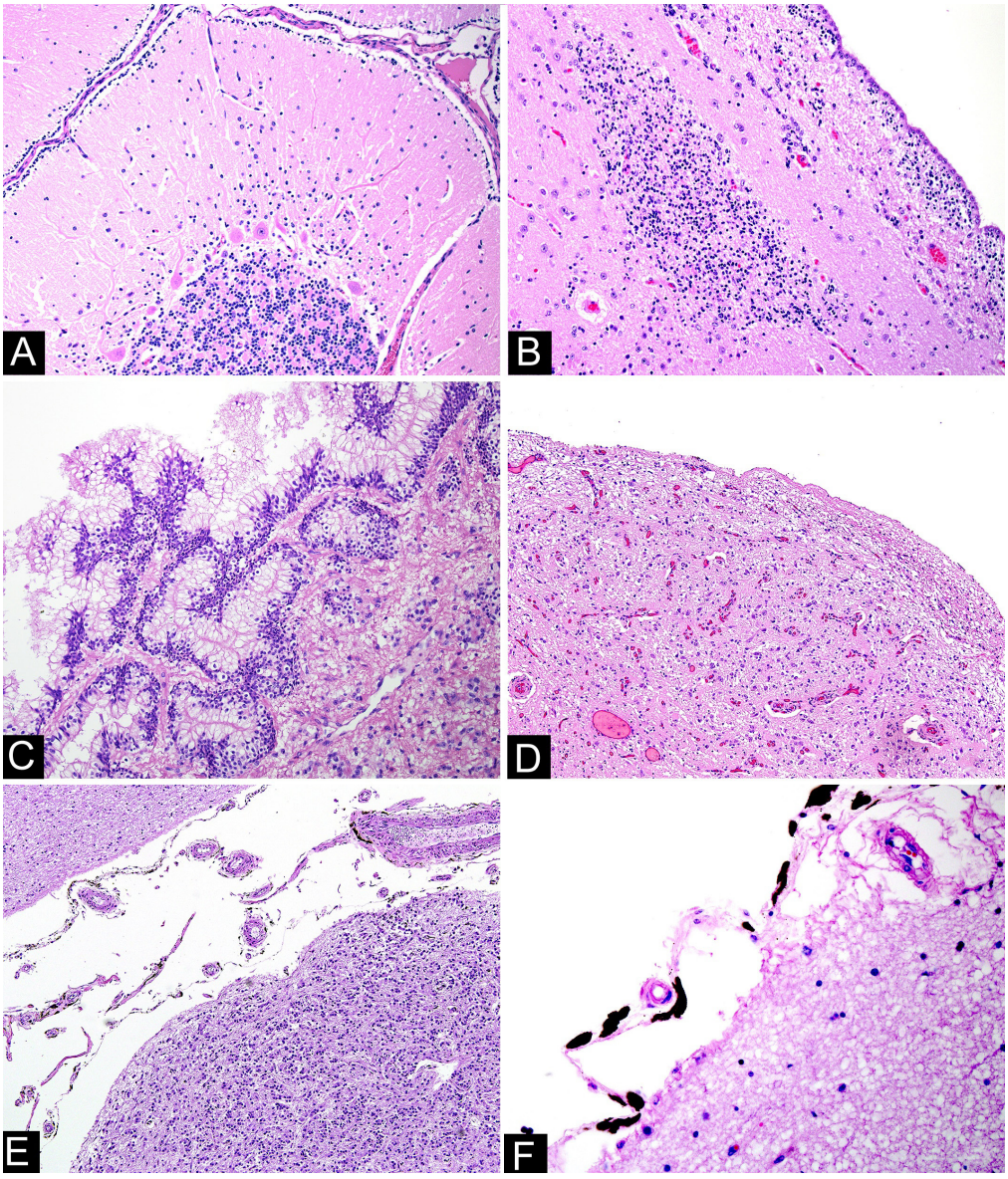

Fig.3. Non-lesions of the bovine brain. (**A**) The external granular cell layer is a mantle of germinative cells found in fetal, neonatal and young animals. Few germinative cells appear in the molecullar layer as they migrate towards the granular cell layer. HE, obj.20x. (**B**) The subependymal plaque consists of multifocal aggregates of germinative cells placed between the ependyma and the parenchyma of the brain at the level of the lateral ventricles. HE, obj.20x. (**C**) The subcomissural organ resembles a glandular arrangement with multiple acini formed by columnar cells with vacuolated cytoplasm and basally located nuclei. HE, obj.20x. (**D**) The area postrema is one of the circumventricular organs, located at the dorsomedial aspect of the medulla oblongata (see Fig.2B). It is composed of neuropil intermixed with the abundant vasculature. HE, obj.20x. (**E**) Histologically, the pineal gland or epiphysis is composed of pinealocytes intermixed with interstitial cells, giving a hypercellular appearance to the parenchyma. HE, obj.10x. (**F**) In leptomeningeal melanosis, the melanocytes are linearly distributed along the leptomeninges and can occasionally extend in the perivascular spaces of the peripheral areas of the cerebral parenchyma. HE, obj.40x.

commissure just caudal to the pineal gland; (b) area postrema, an agglomeration of glial cells and small capillaries located dorsally to parasympathetic nucleus of the vagus at the level of the medulla; and (c) the pineal gland. The subcommissural organ functions to secrete aldosterone (Duvernoy & Risold 2007). Because of the glandular structure seen histologically (Fig.3C), this organ could be erroneously interpreted as an epithelial neoplasm, especially when it is seen in an oblique section of the thalamus. The area postrema is located in the caudal portion of the roof of the fourth ventricle and it is observed microscopically when a section of the medulla oblogata is taken at level of the obex (Fig.3D). In humans, the function of the area postrema has been defined as the vomition center and it is there that chemoreceptors are located that will stimulate vomiting subsequent to intoxication (Borison 1974, Leslie & Osborne 1984). Also, the area postrema functions in cardiovascular control, as the cells here will respond to angiotensin II (Barnes & Ferrario 1980). In histologic sections,

the area postrema can be mistaken for gliosis, or when the capillaries are very prominent, it could be confused with a vascular neoplasm. The pineal gland is an evagination of the dorsocaudal aspect of the epithalamus of the diencephalon, which it is hold through a prolongation that contains the small recess of the third ventricle. The pineal gland is derived from neural epithelium, and consists of glial cells pinealocytes, and blood vessels but contains no true neurons. Pinealocytes synthesize melatonin from serotonine and also contain norepinephrine and thyrotropin releasing hormone (Gartner & Hiatt 2007, Garman 2011). In the lobes of the pineal gland, the pinealocytes are arranged around small lumina (Fig.3E). Neuropathologists prefer the term pineal body to pineal gland, because pinealocytes are neurosensory cells. The capsule and septa of the pineal gland are formed of pia mater and arachnoid, and are continuations of the leptomeninges of the diencephalon, and it is here that the blood vessels penetrate into the pineal (Vigh et al. 1998).

Melanin in the meninges is a non-lesion that can be grossly observed. Histologically, the melanocytes are flattened cells with dendritic cytoplasm and filled with granular dark-brown pigment. These cells can be found in the leptomeninges, or in the vessels within the neuropil, adjacent to the leptomeninges (Fig.3F). In transverse sections, melanocytes can be confused with hemosiderin-laden macrophages. In immunohistochemical studies that utilize diaminobenzidine (dark) as the chromogen, melanocytes can confuse the correct interpretation of the results (Summers et al. 1995).

Lesions of no clinical significance

Lipofuscin is a yellow-brown granular pigment that accumulates in the cytoplasm of neurons in aged animals and apparently does not alter the function of the neuron. It generally deposits in one pole of the perikaryon (Fig.4A), but can also be observed in the cytoplasm of glial cells or free in the neuropil. The extracellular occurrence of lipofuscin can be explained by the process of exocytosis or through rupture of glial cells that contained the pigment. This pigment can be engulfed by macrophages (Jahns et al. 2006a, Wohlsein et al. 2013). Other age-related alterations include mineralization and hyalinization of blood vessels in the brain and meninges (Jahns et al. 2006a, Mandara 2003), and *corpora amylacea* in astrocyte processes. Mineralization of blood vessels in the brain of cattle (Fig.4B) affects predominantly the vessels of the dentate nucleus, globus pallidus, internal capsule, and caudal nucleus, without any association with generalized vascular disease (Wohlsein et al. 2013). *Corpora amylacea* are common in the brains of aging mammals and consist of glucose polymers ("polyglucosan bodies"); they are located within the cytoplasm of astrocytes (Hirano 1985, Garman 2011) and should be differentiated from Lafora bodies, which can also be an age-related change (Yanai et al. 1994, Borras et al. 1999), but these are seen in within neuronal perikaryon and axon (Minassian 2001). Those that are in the axons are difficult to differentiate from *corpora amylacea* because both appear to be free in the neuropil (Wohlsein et al. 2013). Sometimes they can be mistaken for fungal organisms, since they have the same affinity for the special stains used to highlight these organisms.

Concretions of the pineal gland can occur in humans, cattle, horses, sheep, and donkeys, and can vary in size from several microns to several millimeters in diameter. These concretions are called *corpora arenacea*, *acervuli cerebri*, or brain sand (Fuller & Burger 2007). They are formed from concentric dark laminations rich in protein alternating with lighter layers rich in calcium. Mineral structure consists morphologically of hydroxyapatite and carbon apatite, with calcium and phosphorous as the principal constituents. These concretions generally localize between the nervous fibers (axons) and occasionally within glial cells (Fig.4C). The laminations could correspond to fluctuations of the pineal influenced by circadian rhythm or solar intensity (Vigh et al. 1998).

Lesions of no clinical significance can be confused with lesions specific to certain important diseases of the CNS. One of these is vacuolation of the cytoplasm of neurons in the red nucleus of the mesencephalon (Fig.4D) that could be mistaken for a lesion of BSE. It should be remembered that neuronal vacuolation is not always synonymous of BSE. Spongiform alterations of BSE occur in the neuropil (within neuronal processes, especially dendrites) and in the perikaryon of neurons from specific locations in the brainstem. However, spongiform alterations affecting the neuropil and neuronal bodies are also described in natural and experimental cases of rabies in opossums, foxes (Charlton et al. 1987), cattle (Foley & Zachary 1995); in ataxic Rottweiler puppies, (Jardim et al. 1999), and in goats with progressive paresis (Lancaster et al. 1987). Neuronal vacuolation rich in lipids was observed in one raccoon (Hamir & Fischer 1999). Incidental intracytoplasmic vacuolation of neurons in the red nucleus of the mesencephalon is found in 64% of normal adult cattle (Gavier-Widen et al. 2001), but has been seen even in cattle with only 12 months of age (Hamir et al. 2001). Vacuolation of the neuronal perikaryon in the habenular nucleus (Gavier-Widen et al. 2001) can be seen in 50% of normal cattle (Fig.4E). Sporadic neuronal vacuolation has been seen in sheep and goats (Hooper 1999), horses (Jahns et al. 2006a), and swine, (Jahns et al. 2006b), generally in neurons of the brainstem.

Sarcocystis spp. are usually seen distending myofibers of the heart, skeletal muscle, and Purkinje fibers, usually without inciting any inflammatory response (Fig.4F). Cattle become infected through ingestion of sporocysts excreted from the definitive host (carnivores, omnivores), that are in turn infected through ingestion of mature cysts (sarcocysts) in the muscles of the intermediate hosts. Infection by *Sarcocystis* spp. is common in cattle and does not produce lesions because it localizes within endothelial cells. Rupture of the cysts can cause the formation of small granulomas. Cattle are intermediate hosts for three species of *Sarcocysts*: *S. cruzi*, *S. hirsuta*, and *S. hominis*. (Dubey et al. 1988).

Axonal spheroids can be incidental findings, and are most frequently seen in the vestibular, cuneate, and accessory cuneate nuclei (Jahns et al. 2006a). In dogs, the presence of axonal spheroids is correlated with age and they are seen in 50% of dogs more than 12 years of age (Borras et al. 1999). Finding a few axonal spheroids in the brain also is a common incidental finding in sheep and goats, horses, and swine (Hooper 1999, Jahns et al. 2006a,b). Perivascular cuffs can be discrete and nonspecific. Inflammation of this type was seen in about 30% of normal adult cattle (Gavier-Widen et al. 2001). The cause of these inflammatory changes is controversial, but the clinical significance is minimal.

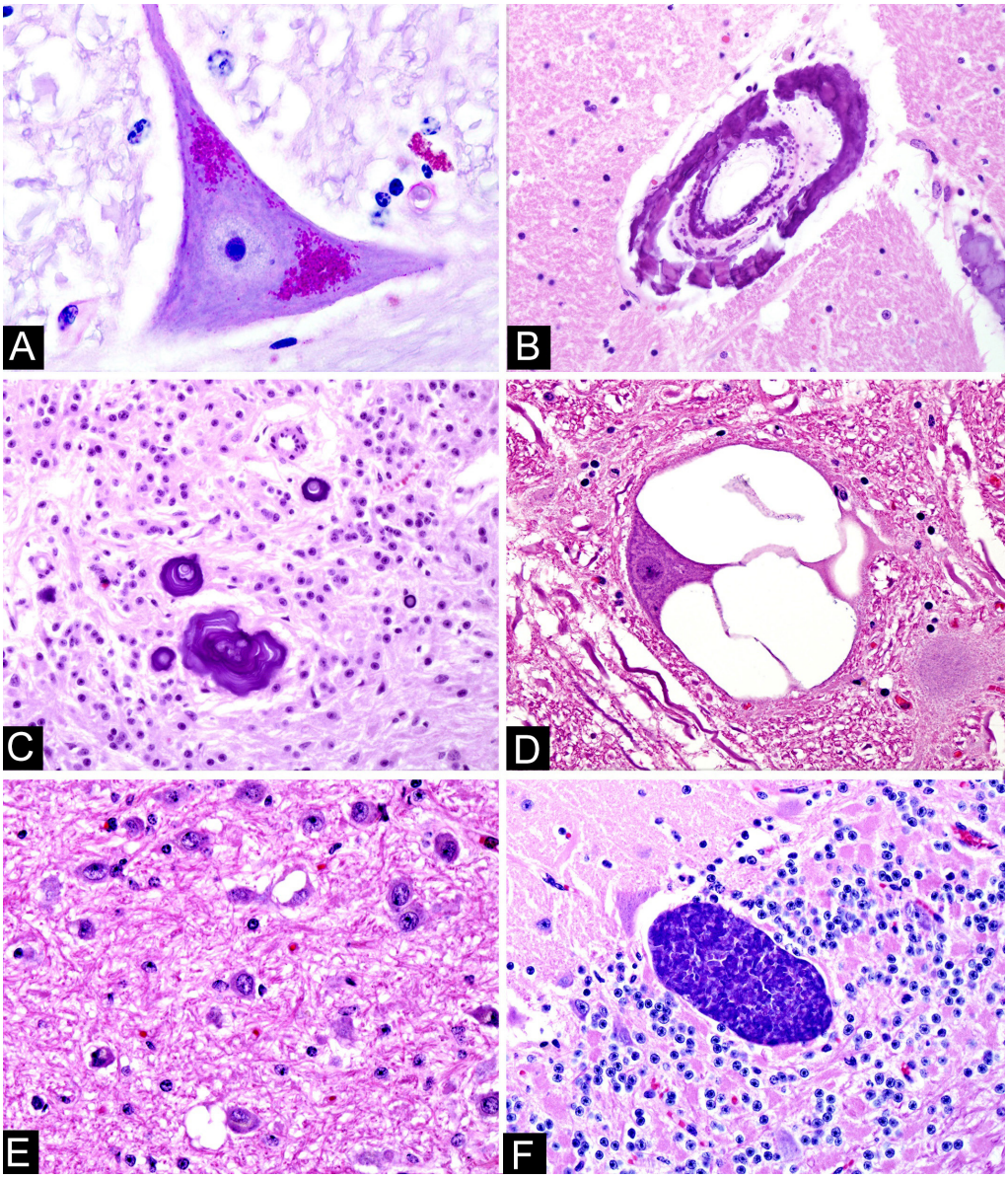

Fig.4. Incidental findings of the bovine brain. (**A**) Lipofuscin is a yellow-orange pigment that taccumulates within the perikaryon of neurons in aged animals. In Periodic acid-Schiff (PAS) stain, it appears as PAS-positive granules. PAS, obj.40x. (**B**) Vascular mineralization (siderocalcinosis) at the level of the internal capsule. HE, obj.20x. (**C**) Pineal gland concretions or corpora arenacea, brain sand or pineal (acervuli) are multiple concentric lamellar basophilic structures within the pineal gland. HE, obj.40x. (**D**) Multiple vacuolations of the perikaryon of a neuron of the red nucleus in the midbrain. HE, obj.40x. (**E**) Neuronal vacuolation of the habenular nucleus in the thalamus. HE, obj.40x. (**F**) Quiescent cyst of *Sarcocystis* sp. in the granular cell layer of the cerebellum. HE, obj.40x.

Artifacts due to the methods of slaughter, sampling, and fixation of the brain

Artifacts due to the method of slaughter, samping, or manipulation of fresh brain could be mistaken for significant antermortem changes. In most cases, these changes are not seen macroscopically. Lesions due to the method of euthanasia can be the cause of death, but often simply mask other alterations that might be a clue as to what was the underlying disease that led to the animals' euthanasia. These include submeningeal hemorrhages (Fig.5A). These lesions should be correctly interpreted as being inflicted on a brain that was normal prior to the decision to kill the bovine. Artifacts related to the collection and manipulation of the brain includes bone particles that lodge within the brain when it is removed from the cranial cavity with a saw, and can mimic pathologic mineralization (Fig.5B). Dark neurons or Cammermeyer neurons (Fig.5C) are a partially controversial subject (Cammermeyer 1978). However, it is generally accepted that it is an artifact caused by postmortem trauma to the nervous tissue caused by handling it prior to fixation (Jortner 2006, Wohlsein et al. 2013). This artifact can also be produced by improper fixation. Dark neurons should not be confused with red neurons, a hallmark neuronal necrosis

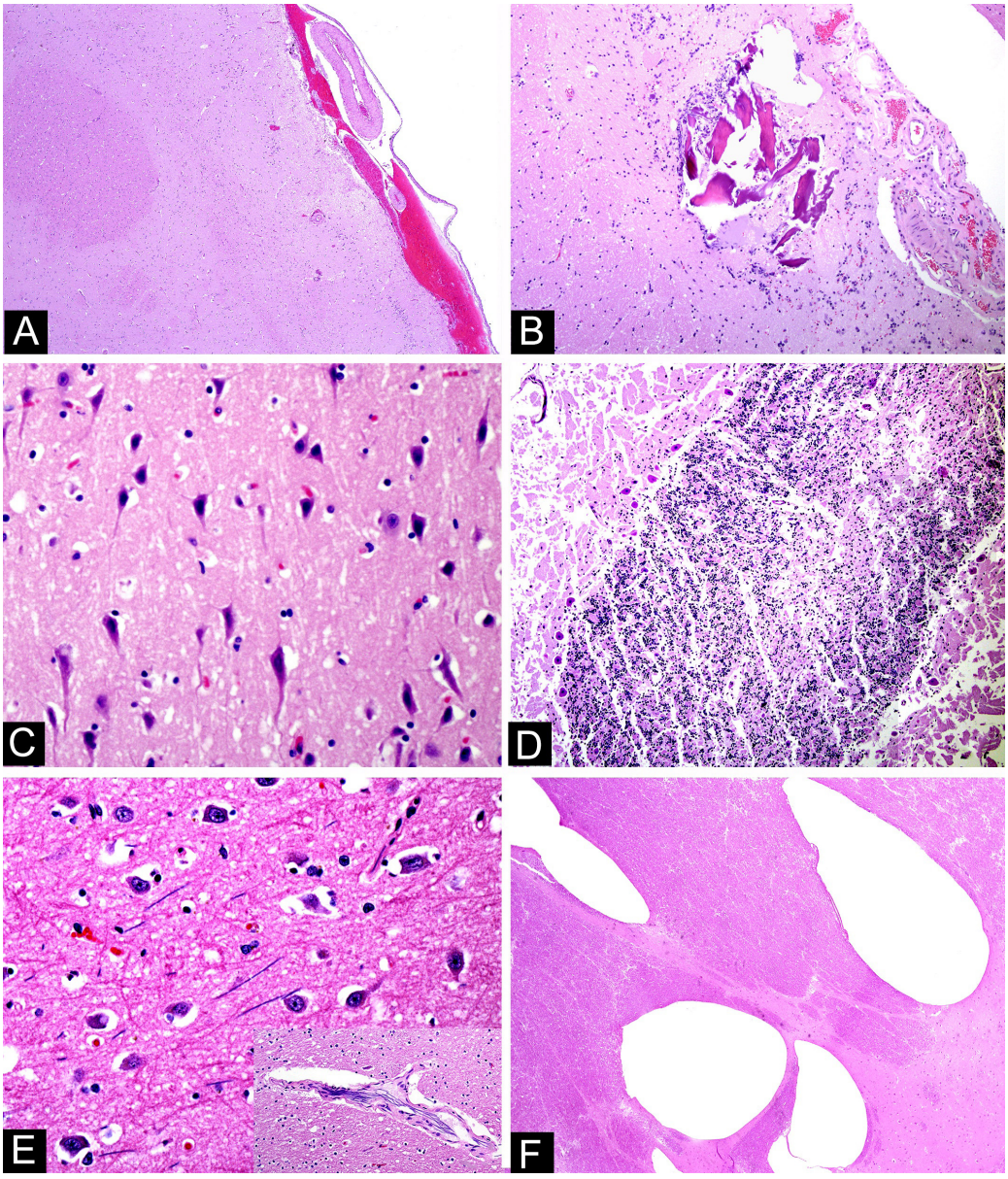

Fig.5. Artifacts in the bovine brain. (**A**) Leptomeningeal hemorrhage due to the use of a captive bolt to stun cattle before death by bleeding. HE, obj.10x. (**B**) A focal aggregate of bone sawdust is within the brain parenchyma. This artifact is commonly found at the lateral areas of the cerebrum when access to the brain is made by open the cranium vault with a handsaw or striker saw. HE, obj.20x. (**C**) Dark neurons or Cammermeyer neurons are neurons in the cerebral cortex with shrunken with dark basophilic cytoplasm and dark condensed nuclei. HE, obj.40x. (**D**) Freezing artifact. Linear interconnecting clefts crisscross the cerebellar cortex. Those clefts are the result of freezing and subsequently thawing of the neural tissue. HE, obj.10x. (**E**) Postmortem artifact. Multiple postmortem bacilli are in the cerebral cortex neuropil without inflammatory reaction. Inset: many bacilli obliterate the lumen of a blood vessel. HE, obj.40x. (**F**) Postmortem artifact. Multiple variable-sized cavities in the brain parenchyma, also called Swiss cheese brain, result from gas bubbles produced by clostridial bacilli. HE, obj.10x.

(Cantile & Youssef 2016). Linear clefts are made by crystals that form when unfixed nervous tissue is frozen (Fig.5D).

Autolysis and putrefaction

When the pathologist receives a brain to be examined in the diagnostic laboratory, there is usually little history regarding the time between the death of the animal and the necropsy and subsequent fixation of tissues. As an organ, the brain is relatively resistant to the process of autolysis, when compared with organs of the abdominal cavity. Autolysis of the CNS can mimic lesions associated with certain diseases, and be confusing to the pathologist. Also, some artefacts observed when the brain is not immediately fixed in formalin after death needs to be differentiated from ante mortem lesions.

Autolysis of gray matter can mimic lesions of trauma and autolytic vacuolar changes in myelin can mimic intramyelinic edema or Wallerian degeneration. Halos around the perikaryon of neurons, oligodendrocytes, and small vessels are common

findings in brains that are autolyzed and should not be confused with edema. Also, as autolysis sets in, glial cells can become small and hyperchromatic. Seeing cadaveric bacilli in the lumen of vessels and dispersed within the nervous tissue can help to determine the extent of autolysis. Clostridial bacilli proliferate extensively in the gastrointestinal tract after death, invade the circulatory system, and colonize various organs, including the brain (Fig.5E). These bacteria may, on occasion, produce gas that distends the nervous tissue making large multifocal vacuoles (Fig.5F).

Autolysis of the granular layer of the cerebellum is an artifact seen only in cattle (Rech et al. 2008) and humans (Albrechtsen 1977, Fuller & Burger 2007). This autolytic change is referred to as cerebellar conglutination, or "etát glace" (Fuller & Burger 2007), and it appears as a pale area in the granular layer. At higher magnification, one can see lysis of the granular neurons, with preservation of Purkinje cells (Rech et al. 2008). Although it is a postmortem phenomenon, it can be confused with necrosis of the granular cell layer caused by ischemia, and it was described in Japan as a specific bovine disease, named there as Kiriyoi disease (Goto et al. 1959, Yamagiwa & Goto 1959). Interestingly these publications (Goto et al. 1959, Yamagiwa & Goto 1959) are cited in some neuropathology texts (Fankhauser & Luginbühl 1968), thus unfortunately validating this true postmortem phenomenon as a real antemortem lesion.

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