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Case Report

Metastatic osteosarcoma as a cause of hemorrhagic stroke in a dog

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Abstract

The aim of this case report is to describe an uncommon hemorrhagic cerebrovascular accident (CVA) associated with an osteosarcoma (OSA) metastasis. Cerebrovascular accident (CVA) is the acute onset of a neurological deficit from any change in blood supply resulting from a pathological process, characterizing a stroke and among all the causes, the neoplastic thrombus of osteosarcoma, specifically, is the most infrequent in clinical practice. A seven-year-old female midsize mixed-breed dog was submitted to a forelimb amputation for showing an osteoblastic OSA in proximal humerus. Three weeks later, the patient presented uninterruptible seizure and coma, resulting in death. At necropsy, it was observed a friable and reddish mass in the left frontal cortex which, microscopically was constituted by OSA metastasis in association with multiple ruptures of blood vessels and focally extensive severe bleeding, which caused a CVA. At immunohistochemistry, neoplastic cells of the humerus and the brain metastasis were positive for vimentin and osteonectin, confirming the diagnosis of osteoblastic OSA. The reports about OSA metastasis on the brain are rare in animals and humans, what could be related to the low frequency and few diagnosis ante and post-mortem. As uncommon as the description of OSA metastasis to the brain, is the CVA associated to them, demonstrating that reporting cases related to these clinical and anatomopathological lesions are important for the scientific community.

Key words: brain, neoplasia, intracranial hemorrhage, pathology.

Introduction

Cerebrovascular accident (CVA) is the acute onset of a neurological deficit from any change in the blood supply resulting from a pathological process (5, 11). There is no scientific data to quantify the incidence of strokes in dogs. In humans, it is described that 88% of all strokes are ischemic, the other 12% comprise intracerebral and subarachnoid hemorrhages (2, 21). Strokes in cerebrospinal arteries are slightly less common in animals than in humans and the main causes of this include trauma, diabetes mellitus, hypothyroidism, bacterial plungers, hypertension, vascular malformation, and coagulopathy, other less frequent causes include parasites, neoplasia, cerebral amyloid angiopathy and necrotizing vasculitis (1, 21).

Before the advent of magnetic resonance imaging (MRI) and computerized tomography, many of the cases of CVA in dogs came from a postmortem diagnosis, a complicating factor in the development of incidence

studies, since only animals in a serious clinical state would be euthanized. Neoplasms have a much less favorable prognosis than various diseases and in many cases, euthanasia has been a choice. For this highly debilitating characteristic, most of the cases of CVA in the veterinary literature are associated with malignancies, mainly intravascular angiocentric lymphomas. (5, 6, 8).

Currently, image techniques have now become a reality applicable in day-to-day and the identification of cases of stroke became more frequent (5). Based on this information, it is understood that, unlike in humans, where the diagnosis of stroke occurs before the death and is often treated, thromboembolism in animals has a late diagnosis, after death, usually associated with a neoplastic cause (2, 6).

Osteosarcomas are the most common bone neoplasms in animals and humans (2, 12). In dogs correspond to 85% of tumors of the appendicular skeleton and 75% of all bone tumors, focusing mainly in midsize to large male dogs, of all ages, with a higher occurrence between 7 and 8 years of age (7, 18, 20). It does not appear to be hereditary, although breed predilection like Great Dane, German Shepherd, St. Bernard, Irish Setter, and Boxer may occur. There is evidence that Rottweilers and Greyhounds have a high rate of disease occurrence (12, 17). The treatment protocols for this neoplasm include radical surgery and chemotherapy, however, despite the treatment, these tumors usually cause death as a result of local or distant infiltration from the primary focus; an even worse prognosis is observed when lymph node metastasis is present, and studies indicate that 5 out of 200 dogs with OSA already have infiltration at this site at the time of amputation (14).

It is estimated that 30% of the human patients with any kind of cancer develop brain metastasis, however, only 0,8% of them all come from musculoskeletal tumors (osteo and rhabdomyosarcoma) or from soft tissue sarcomas, in this way, the metastasis of OSA to central nervous system is considered rare in all species, independent of primary origin be bone or extraosseous (12, 16, 19). Despite the rarity even in dogs, Rottweilers are known to have more chances of having brain metastases than other breeds (12). The aim of this work is to report the occurrence of a CVA associated with an OSA metastasis in a dog.

Case report

A 7-year-old intact female mixed-breed dog was referred to the veterinary hospital of Santa Catarina State University (Brazil) due to an increase of volume in the left proximal humerus, resulting in difficulty of locomotion. The radiologic evaluation showed classic features of bone neoplasia: Codman's triangle, osteolysis and periosteal palisade reaction, and an increase in adjacent soft tissues compatible with osteosarcoma (18). Complete limb amputation was performed four days after diagnosis. At gross evaluation, a firm to hard, whitish mass, measuring 15x20 cm was occupying all the proximal and diaphyseal region of the humerus, which extended to the humerus-radio ulnar joint (Fig. 1A).

Histopathological evaluation showed a non-limited mesenchymal malignant neoplastic proliferation, organized in multiple nests and bundles of fusiform to starry shaped cells with an osteoid matrix, sometimes forming a lace-like pattern or irregular islands, sometimes filled by erythrocytes, lined by a moderate quantity of multinucleate giant cells. Neoplastic cells had vesicular nuclei, showing 1 to 3 nucleolus, boundless cytoplasm, weakly basophilic and occasionally finely eosinophilic granular material in the periphery. Pleomorphism was accentuated, and 4 mitotic figures/10 high-power field (40x) were observed. This neoplasm was characteristic of an osteoblastic osteosarcoma (Fig. 1B).

The patient returned clinically well eight days after surgery presenting a good healing process. However, thirty-six days after the last clinical evaluation, the patient returned comatose, anorexic, with a history of multiple seizures per day, sometimes uninterrupted (Status epilleticus). The patient was maintained on fluid therapy with glycoside solution and the seizures were controlled by the administration of diazepam and phenobarbital, but without conscious recovery. The dog died in the thirty-seventh day after the surgery.

During necropsy, the brain showed moderate asymmetry in the left hemisphere, with no changes in gyri or color. After serial cross-section of the brain, a friable mass of 2 cm in diameter, red and with discrete fibrinous delimitation in the frontal cortex region, was found just above the rostral portion of the corpus callosum, involving grey and white matters (Fig. 2). In the histological analysis, we observed neoplastic proliferation not bounded or circumscribed, expanding the grey and white matters, composed by mesenchymal cells organized in multiple nests or bundles of cells forming a dense sheet pattern. These cells varied from spindle to stellate-shaped, with central and vesicular nuclei, 1 to 3 nucleoli and occasional macronucleolus. The cytoplasm showed projections which followed to multiple directions, weakly basophilic and, sometimes, discrete peripheral production of the osteoid matrix was evident. There were an intense anisocytosis and anisokaryosis and 3 mitotic figuress/10 high-power field (40x).

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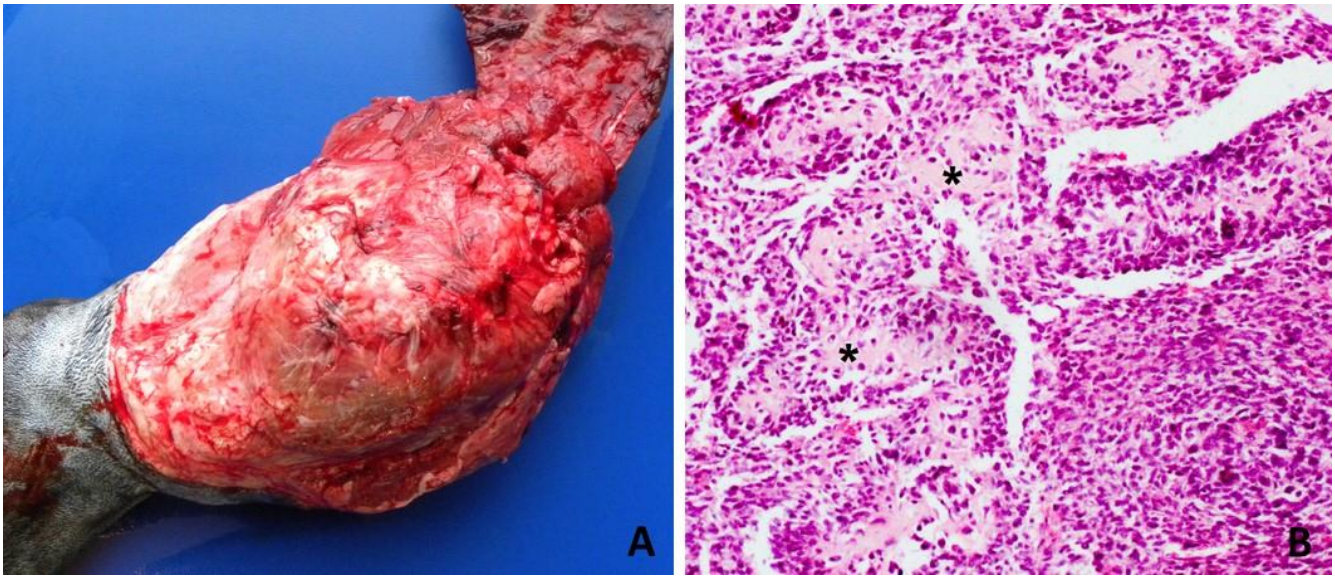


Figure 1. Primary canine osteosarcoma in the humerus. **A.** Side view of the limb, after dissection with firm, tough and whitish mass, measuring 15x20 cm in epiphysis and diaphysis of the humerus. **B.** Photomicrograph of a canine primary humerus osteoblastic osteosarcoma. Proliferative mesenchymal cells connected by a thin fibrovascular stroma, organized in multiple nests or bundles of cells with peripheral production of an osteoid matrix (*). HE. 40x obj.

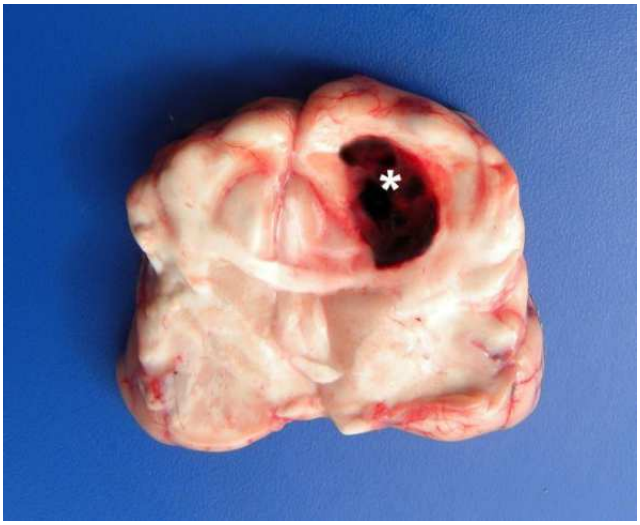


Figure 2. Brain osteosarcoma metastasis: a transversal section with a friable reddish mass of 2 cm in diameter (*) in the left frontal cortex.

Neoplastic cells were evidenced in association with the tunica intima of a blood vessel, and multiple blood vessels adjacent to the metastatic neoplasm showed loss of integrity of all tunica (Fig. 3A). Due to this, in the margins of neoplastic tissue, there was a focally extended and severe hemorrhage, which infiltrated profusely in the neuropil and dissociated the brain, corroborating to the CVA focal areas of necrosis accompanied lymphocytes, plasma cells, and few Gitter cells infiltrates. At necropsy was also observed multiple nodules in the lungs, with 1 to 4 cm in diameter, firm and whitish, comprehending not more than 5% of all parenchyma. Microscopically, these nodules characterized metastasis of osteosarcoma.

Immunohistochemistry (IHC) was performed in humerus and brain neoplasm for vimentin (dilution 1:800, clone V9, Invitrogen, U.S.A.), osteonectin (dilution 1:200, clone 15G12, Kamiya Biomedical, U.S.A.), pan-cytokeratin (dilution 1:2000, clones cocktail AE1+AE3, Dako Cytomation, U.S.A.) and glial fibrillary acidic protein - GFAP (dilution 1:1000, clone GA-5, Zeta Corporation, U.S.A.) with 10mM pH6 citric-acid solution (pressure cooker/3 minutes) for antigen retrieval, as well as amplified with third generation polymer, conjugated with peroxidase enzyme (Reveal Polyvalent HRP, Spring Biosciences, U.S.A.), using diaminobenzidine (DAB, Sigma Aldrich, U.S.A.) as chromogen. As a result, there was diffuse immunostaining in the neoplastic cells of the humerus and brain metastasis for vimentin and osteonectin (Fig. 3B) and negative staining for cytokeratin and GFAP.

Discussion

Although the most recent cancer literature in veterinary medicine includes the brain as the site of metastases of OSA (8), the scarcity of reports in the main databases still leads us to agree with previous literature that indicate the rarities of these cases (13). A retrospective study of extraskeletal OSA (OES) in 169 dogs and a comparative evaluation of OSA in dogs and humans described metastasis in lymph nodes, lungs, kidneys, spleen, bone marrow, tegument, but did not mention the brain (9, 14). In another study of OSA in 156 dogs, brain metastasis was noticed only in the Rottweiler, what

suggests that biological behavior of this breed may be different (12).

A case of brain metastasis of OES was reported in a dog that presented shock, tetraparesis, right head tilt, nystagmus and mydriasis, loss of facial sensibility and hyporeflection of all joints. Neoplastic masses were observed in multiple organs and brain was infiltrated in the temporal region. The neoplasm was associated with the vessel wall, promoted vascular obstruction besides the multiple areas of liquefaction necrosis, because of the ischemic CVA (16). The association of the brain tumor with the vessel wall is a similar factor in both cases, however, in the present report, we found a loss of continuity on the wall of the vessel, promoting a hemorrhagic CVA.

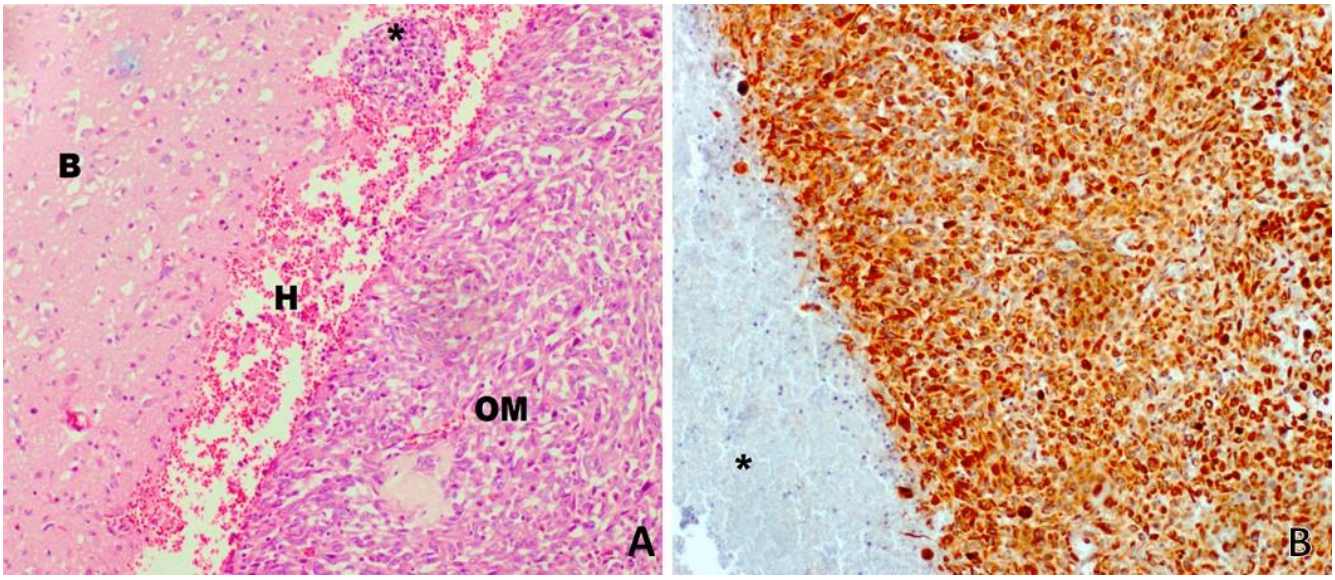


Figure 3. Brain osteosarcoma metastasis. **A.** Cerebral hemorrhage in tumor interface (H), with complete obliteration of blood vessel by a neoplastic thrombus (*). Arrowhead: Endothelial cells. HE. 40x obj. **B.** Immunohistochemistry. The cytoplasm of neoplastic cells stains strongly for osteonectin. Note negative nervous tissue (*). 40x obj.

In human medicine, brain metastasis is common, especially with primary lung tumors, such as small cell carcinoma (4). These metastases occur due to the activation of WNT/TCF pathway and the dynamics of the cerebral vascularization, where the adhesion of neoplastic cells to the endothelium can occur when circulating neoplastic cells reach regions where the lumen of the capillaries is narrower and blood flow slows (4, 15). Unlike the case cited above, in this case, no organ, other than the lung demonstrated metastasis. Considering this information, cerebral metastasis could be attributed to primary metastasis in the lungs, from where the plunger drifted into the bloodstream and adhered in the lumen of small, slow-flowing capillaries, generating a thromboembolism.

Coma is commonly associated with injuries to the brainstem or cerebral cortex, and seizures are closely related to lesions in the region of the frontal cerebral cortex (3, 11). In this report, the patient returned to the hospital unresponsive to painful stimuli or producing reflexes in a profoundly comatose condition, not recovering consciousness at any time, demonstrating sequential and intense seizures.

The association of clinical signs induced the suspicion of lesions in the frontal cortex region, which included OSA metastasis, in addition to possible brain stem compression. Although tumor growth promoted asymmetry between the hemispheres, macroscopic signs of cerebral compression by neuropil distension were not evident during necropsy. No gross and histopathological changes were observed in the structures of the brainstem that corroborated with the comatose condition.

Cancer is considered a pre-thrombotic permanent state due production of hyper coagulants factors which act, as a predisposing thrombus formation that may bind to vessel walls after cytochemical stimuli and generate vascular accidents of several complexities (10). The production of coagulation activation factors, mainly VEGF-A in OSA, along with the vascular lesion concomitant to the growing of the metastatic tumor has great importance in the occurrence of hemorrhagic vascular lesions, independent if the patient is in the initial or advanced stage of the disease (19). It is believed that this characteristic of intravascular hypercoagulability has favored the adhesion of the neoplastic embolies to the vessel wall. The intravascular conditions are favorable to the growth and expansion of the tumor, which would have caused the rupture of the vascular tunics, and as a consequence, the vascular accident.

In the literature, there are few reports that point to the occurrence of CVA, mainly associated with metastases in the frontal lobe, however, there are reports of cats with

ischemic frontal lobe accidents associated with erratic migration of Cuterebra fly larvae, showing consciousness depression, circling and blindness, where the neurological disease lasted from 7 days to 2 weeks (22).

In a retrospective study conducted through medical records of 40 dogs, that evaluate clinical and topographic characteristics of suspected stroke, 11 lesions were found in the telencephalon, of which 2 cases of CVA were found in the territory of the Rostral cerebral artery. These patients showed consciousness depression, contralateral nasal hyperalgesia and contralateral postural and threat reflex deficits (5).

Based on this study, a hypothesis to the AVC is that the migration of the neoplastic embolies occurred through the rostral cerebral artery to adhere to the lumen of its deeper ramifications, to grow and to promote rupture of the vessel, generating a vascular accident. There was no clinical evaluation of postural or menace reactions in the case reported here, since the animal was not conscious at any moment, however, it is believed that the clinical presentation is directly related to the site where the bleeding occurred and the intensity of this lesion.

Greater investigation in animals with OSA with concomitant central neuropathy are required to exclude brain metastasis and CVA. A clinical investigation using imaging methods such as MRI, for example, favors the determination of the patient's prognosis, allowing for the targeting of a medical conduct aimed at maintaining the animal comfort and, when surgical intervention is possible, drawing up a surgery plan to give greater support to the surgeon. Although a complete neurological evaluation may help to determine the site of brain injury, the aid of the MRI should be requested whenever possible for better acuity.

Even though the histological characteristics of the brain metastasis have returned to the primary humerus OSA, immunohistochemically evaluation was essential to accurately determine the origin of the neoplastic cells. As atypical as the description of OSA metastasis to the brain, are the CVA associated with it, demonstrating that cases related to these clinical signs and anatomopathological lesions are important for the scientific community, expanding and composing a scarce database.

References

1. Cantile C, Youssef S. Nervous System. In: Maxie MG, editor. Jubb, Kennedy and Palmer's Pathology of Domestic Animals. Saint Louis: Elsevier; 2016. p. 250-406.
2. Casals JB, Pieri NCG, Feitosa MLT, Ercolin ACM, Roballo KCS, Barreto RSN, Bressan FF, Martins DS, Miglino MA, Ambrósio CE. The Use of Animal Models for Stroke Research: A Review. Comp Med. 2011;61(4):305-13.
3. De Lahunta A, Glass E. Veterinary Neuroanatomy and Clinical Neurology. 3th ed. Saint Louis: Elsevier; 2009. p. 454-75.
4. Eichler AF, Chung E, Kodack DP, Loeffler JS, Fukumura D, Jain RK. The biology of brain metastases - Translation to new therapies. Nat Rev Clin Oncol. 2011;8(6):344-56.
5. Garosi L, McConnell JF, Platt G, Barone JC, Baron A, De Lahunta A, Schatzberg SJ. Clinical and topographic magnetic resonance characteristics of suspected brain infarction in 40 dogs. J Vet Intern Med. 2006;20(2):311-21.
6. Garosi L. Cerebrovascular diseases in dogs and cats. Vet Clin North Am Small Anim Pract. 2010;40(1):65-79.
7. Heyman SJ, Diefenderfer DL, Goldschmidt MH, Newton CD. Canine axial skeletal osteosarcoma: a retrospective study of 116 cases (1986 to 1989). Vet Surg. 1992;21(4):304-10.
8. Higgins RJ, Bollen AW, Dickinson PJ, Sisó-Llonch S. Tumors of The Nervous System. In: Meuten, DJ, editor. Tumors in Domestic Animals. 5th ed. New Jersey: John Wiley & Sons; 2017. p. 834-91.
9. Langenbach A, Anderson MA, Dambach DM, Sorenmo KU, Shofer FD. Extraskeletal osteosarcomas in dogs: a retrospective study of 169 cases (1986-1996). J Am Anim Hosp Assoc. 1998;34(2):113-20.
10. Lee AY. Thrombosis in cancer: an update on prevention, treatment, and survival benefits of anticoagulants. Hematology Am Soc Hematol Educ Program. 2010:144-9.
11. Lorenz MD, Kornegay JN. Handbook of Veterinary Neurology. 4th ed. St. Louis: WB Saunders Company; 2011. p. 297-322.
12. McNeill CJ, Overley B, Shofer FS, Kent MS, Clifford CA, Samluk M, Haney S, Van Winkle TJ, Sorenmo KU. Characterization of the biological behavior of appendicular osteosarcoma in Rottweilers and a comparison with other breeds: a review of 258 dogs. Vet Comp Oncol. 2007;5(2):90-8.
13. Morello E, Martano M, Buracco P. Biology, diagnosis and treatment of canine appendicular osteosarcoma: similarities and differences with human osteosarcoma. Vet J. 2011;189(3):268-77.
14. Mueller F, Fuchs B, Kaser-Hotz B. Comparative biology of human and canine osteosarcoma. Anticancer Res. 2007;27(1A):155-64.
15. Nguyen DX, Chiang AC, Zhang XHF, Kim JY, Kris MG, Ladanyi M, Gerald WL, Massagué J. WNT/TCF signaling through LEF1 and HOXB9 mediates lung adenocarcinoma metastasis. Cell. 2009;138(1):51-62.
16. Pazzi P, Tompkins S, Kirberger RM. Canine spirocercosis-associated extraskeletal osteosarcoma with central nervous system metastasis. J S Afr Vet Assoc. 2013;84(1):E1-4.
17. Rosenberger JA, Pablo NV, Crawford PC. Prevalence of and intrinsic risk factors for appendicular

osteosarcoma in dogs: 179 cases (1996–2005). J Am Vet Med Assoc. 2007; 231(7):1076-80.

18. Schulz, K. Other diseases of bones and joints. In: Fossum TW, editor. Small Animal Surgery Textbook. Philadelphia: WB Saunders Company; 2008. p. 1333-56.
19. Shweikeh F, Bukavina L, Saeed K, Sarkis R, Suneja A, Sweiss F, Drazin D. Brain metastasis in bone and soft tissue cancers: A review of incidence, interventions, and outcomes. Sarcoma. 2014;ID475175:1-19.
20. Spodnick GL, Berg J, Rand WM, Schelling SH, Couto G, Harvey HJ, Henderson RA, MacEwen G, Mauldin N, McCaw DL, Moore AS, Morrison W. Prognosis for dogs with appendicular osteosarcoma treated by amputation alone: 162 cases (1978 - 1988). J Am Vet Med Assoc. 1992;200(7):995-9.
21. Wessmann A, Chandler K, Garosi L. Ischemic and hemorrhagic stroke in the dog. Vet J. 2009;180(3):290-303.
22. Williams KJ, Summers BA, De Lahunta A. Cerebrospinal cuterebriasis in cats and its association with feline ischemic encephalopathy. Vet Pathol. 1998;35(5):330-43.