Internal Medicine

Diagnosis of meningeal melanomatosis in a dog using magnetic resonance imaging and cerebrospinal fluid findings

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ABSTRACT. A 13-year-old spayed female Labrador Retriever was presented with severe progressive tetraparesis. The neuroanatomic localization was the C1–C5 spinal cord segments with brainstem or cranial nerve involvement. Magnetic resonance imaging revealed diffuse T1-weighted and T2-weighted hyperintense lesions with strong contrast enhancement spreading through meninges of the cervical spinal cord and the brain. Few small round areas showing T1-weighted hyperintensity and T2-weighted hypointensity were scattered within the lesions. Cerebrospinal fluid analysis revealed neoplastic round cells and possible melanocytes. Malignant melanoma was suspected. At necropsy, the brain and the entire spinal cord were covered with thick, dark membranous tissue. Based on histopathologic findings, a positive response against Melan-A, and no melanoma identified outside the central nervous system, primary meningeal melanomatosis was diagnosed.

KEY WORDS: central nervous system, melanoma, neoplasm, T1 hyperintensity, T2 hypointensity

Pigment deposition in the central nervous system (CNS) of animals manifests as varied entities, ranging from normal meningeal melanosis in black-skinned pigs or black-faced sheep to malignant melanoma and meningeal melanomatosis [28]. Besides primary melanocytic tumors and metastatic melanoma, other CNS neoplasms may also undergo melanization, such as schwannomas, medulloblastomas, and gliomas [28]. Primary melanocytic lesions of the CNS are thought to arise from leptomeningeal melanocytes derived from the neural crest. These could be of four types: melanocytoma, melanoma, melanocytosis, and melanomatosis. Melanocytoma and melanoma are solitary lesions while melanocytosis and melanomatosis have diffuse meningeal involvement [19]. These two pairs of nomenclature represent the benign and malignant ends of a spectrum. Melanocytosis is caused by the abnormal proliferation and melanin production of leptomeningeal melanocytes, and melanocytomas are solitary low grade lesions. Both of them do not invade the surrounding brain parenchyma. On the contrary, melanoma and melanomatosis showed malignant cytological features including mitotic figures and anaplastic, pleomorphic cells. Tissue invasion, hemorrhage or coagulative necrosis may be seen histologically [20]. Primary meningeal melanomatosis is also referred to as a meningeal variant of primary malignant melanoma, and is extremely rare in human medicine [28].

In veterinary literature, only two canine melanoma cases have been reported of having leptomeningeal involvement of disseminated melanoma. Melanoma was found in one dog’s bone marrow, lung, lymph nodes, intercostal muscle, and adrenal gland, along with diffuse leptomeningeal involvement. Based on the lesion distribution, primary leptomeningeal melanomatosis with multiorgan metastasis was suspected [16]. The other dog with uveal melanoma extending into the skull had an apparent primary site of the tumor [8]. In addition, magnetic resonance imaging (MRI) features of meningeal melanomatosis in dogs have not been reported, presumed due to its rarity. This report describes a canine case of primary meningeal melanomatosis, focusing on the clinical, MRI, and cerebrospinal fluid (CSF) features of this rare disease.

A 13-year-old spayed female Labrador Retriever dog was referred due to one-month history of progressive tetraparesis. Pelvic limb weakness was noted initially. Two weeks prior to the referral, the dog started to drag its thoracic limbs, with neurologic signs deteriorating drastically since then. MRI of the cervical spine was performed in a private imaging clinic with an inconclusive diagnosis of inflammatory disease or neoplasia. A CSF tap at the cerebellomedullary cistern site could not be performed successfully at the time.

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Results of the physical and blood examinations were unremarkable. Upon neurologic examination, the dog was alert with a mild right-sided head tilt and non-ambulatory tetraparesis. Delayed or absent postural reactions and decreased voluntary movements were observed in all four limbs. Cranial nerve examination revealed mildly decreased left-sided facial sensation. Results of the rest of neurologic examination were unremarkable. These findings indicated multifocal lesions, primarily involving the C1–C5 spinal cord segments but also the brainstem or cranial nerves. Due to insufficient information obtained from the previous investigation and suspicion of intracranial involvement, MRI and CSF examinations were suggested to be repeated. On the day of the investigations, the neurologic status further deteriorated to only minimal voluntary movements preserved. Bilateral third eyelid protrusion and mild anisocoria were also noted.

Using a 0.2-Tesla unit (Vet-MR, Esaote, Genova, Italy), MRI of the brain and the cervical spine were performed. The protocol included spin echo T2-weighted (T2W; TR 2,800 msec; TE 90 msec), spin echo T1-weighted (T1W; TR 690–800 msec; TE 26 msec), and post-contrast T1W sequences in three orthogonal planes, and fluid-attenuated inversion recovery sequence (FLAIR; TR 6,000 msec; TE 80 msec; TI 1,500 msec) in the transverse plane. A diffuse and infiltrative lesion was found extending along the meninges of the cervical spine and the brain. In the region over the craniocervical junction to the mid-C3 vertebra, the lesion had wrapped around the spinal cord irregularly, and the edge between the lesion and the spinal cord was blurry. In sagittal images, a normal signal of the CSF at the cerebellomedullary cistern could no longer be observed and was replaced by the lesion (Fig. 1). Within the calvarium, the lesion spread from the mid-line to mainly around the right hemisphere of the cerebellum and cerebrum. It infiltrated into the right occipital lobe and coursed cranially along the splenial sulci, affecting the splenial and cingulate gyri bilaterally (Fig. 2). The lesion was generally hyperintense in T1W and T2W images. However, few small areas in the right cerebrum showed T1W hyperintensity and T2W hypointensity. After intravenous gadopentetate dimeglumine injection (Magnevist, Bayer, Berlin, Germany), the lesion was strongly enhanced in T1W images. In addition, there was meningeal enhancement of the entire cervical region and the ventral and right dorsolateral aspect of the brainstem. Moreover, contrast enhancement at the dorsal part of the third ventricle and the junction between the right lateral and the third ventricle were also noted.

Considering the extensive change of the subarachnoid space at the cerebellomedullary cistern, the CSF sample was collected from the lumbar region. The CSF protein content was beyond the processing capacity of the biochemistry analyzer (>400 mg/dl; reference range, 0–45 mg/dl). An increased nucleated cell count of 148 cells/µl (reference range, 0–5 cells/µl) and a mildly elevated RBC count (470 cells/µl; reference range, 0 cells/µl) were detected. Cytology revealed that most of the nucleated cells were round cells, with a few cells containing dense dark gray cytoplasmic granules which were highly suspected to be melanin (Fig. 3). Mitotic figures were also noticed in some round cells. The distribution of nucleated cells was as follows: 78% round cells (including round cells with pigment granules), 12.5% lymphocytes, 6.5% monocytes, 0.5% neutrophils, and 2.5% macrophages.

![Fig. 1. Magnetic resonance imaging of the brain and the cranial cervical spine. T2-weighted (A and B), T1-weighted (C and D), and post-contrast T1-weighted (E and F) images. In the sagittal images (A, C, and E), the lesion extends from C1 to C2 and presents as T2-weighted and T1-weighted hyperintensity with marked contrast enhancement. Normal signal of the cerebrospinal fluid is replaced by the lesion. In the transverse images of the spinal cord at the C1 and C2 junction (B, D, and F), the spinal cord is found to be tightly folded around and pushed to the right side by the lesion.](image-url)
Based on the MRI and CSF findings, malignant CNS melanoma was suspected. Since no definitive treatment was available for diffusive CNS melanoma, an anti-inflammatory dose of prednisolone was prescribed for palliation. The patient’s neurologic signs continued to progress and it passed away within two months from the disease onset. The consent to perform full necropsy was given by the owner.

Upon postmortem examination, the gross lesions were found to be restricted to the CNS. The meninges of the entire brain and the spinal cord had a gray to black discoloration. After proper fixation in 10% neutered formalin, serial cross-sectioning of the brain and spinal cord revealed multifocal to coalescing, gray to black substances occupying and expanding along the cerebral fissures and sulci. Similar features were noticed at the subdural or subarachnoid space of the spinal cord (Fig. 4). Histopathologically, clusters of variably-shaped neoplastic cells with multifocal hemorrhage occupied the leptomeninges and the Virchow-Robin space throughout the entire spinal cord and the brain (Fig. 5). These neoplastic cells were highly pleomorphic (round, polygonal, or spindle-shaped). Some neoplastic cells exhibited small to moderate amounts of brownish to black melanin granules in the cytoplasm. Using immunohistochemistry staining, most amelanotic cells were positive against Melan-A. The nuclei of neoplastic cells were round to oval, often with finely coarse to stippled chromatin and sometimes with single to multiple conspicuous nucleoli.

**Fig. 2.** Magnetic resonance imaging of the brain at two levels: the occipital lobes (A–C) and the third ventricle (D–F). T2-weighted (A and D), T1-weighted (B and E), and post-contrast T1-weighted (C and F) images. Infiltration of the lesion into the cerebral parenchyma is more extensive in the right occipital lobe (A–C). The lesion follows the sulci and extends cranially (D–F). Magnetic resonance imaging characteristics are similar to those of shown in Fig. 1. However, a few small T2-weighted hypointense spots (arrow) are seen, indicating the presence of melanin or hemorrhage.

**Fig. 3.** Cytologic analysis on the cerebrospinal fluid collected from the lumbar tap. Most of the nucleated cells are large round cells with abundant cytoplasm (arrowheads) and some cells have mitotic figures (arrow) (A). Intracytoplasmic dark gray granules accumulation (open arrow) is observed occasionally (B). Wright Giemsa.
Multifocally, there were also mild to moderate leptomeningeal infiltrates and perivascular cuffing of lymphocytes and plasma cells. The morphologic diagnosis was melanomatosis with mild to moderate lymphoplasmacytic meningoencephalitis. The condition was thought to be primary meningeal melanomatosis since no other primary melanoma was identified during necropsy.
In our case, neoplastic cells were only found in meninges of the brain and the spinal cord following a thorough whole-body search. Therefore, a diagnosis of primary meningeal melanomatosis was made. Distinguishing primary CNS melanoma from metastatic melanoma on imaging alone is difficult. Evidence in human medical literature suggests, in addition to the absence of malignant melanoma outside the CNS, several imaging characteristics may increase the suspicion of primary CNS melanoma. These include (1) leptomeningeal involvement, (2) intramedullary spinal lesions, (3) hydrocephalus, (4) tumor in the pituitary or pineal gland, and (5) a single intracerebral lesion [14]. Our case demonstrates the diffuse meningeal involvement of the entire CNS. In human medicine, meningeal melanomatosis is extremely rare and involves a high degree of malignancy. The incidence of this disease is 0.005 in 100,000 people [9]. The rapid clinical progression and diffuse involvement of the entire CNS in our case reflects the malignancy of the disease.

The MRI findings in our patient showed distinctive T1W hyperintensity of the melanocytic neoplasm. The hyperintensity of T1W images was due to the paramagnetic effect of melanin. It has a high affinity for metal ions and serves as a scavenger of organic free radicals [6]. The paramagnetic effects of these ions shorten T1 and T2 values, and thus typically melanomas show T1W hyperintensity, T2W hypointensity, FLAIR hyperintensity, and enhance homogenously with gadolinium [20]. Besides the typical findings of CNS melanoma, three other subtypes have been proposed in human medicine according to tumor melanin content. These subtypes are as follows: (1) non-melanoma characterized by T1W hypo- or isointensity and T2W hyper- or isointensity, (2) uncertain or mixed characterized by mixed MRI signals, and (3) hemorrhagic lesions characterized by different stages of hemorrhage on MRI [19]. In our case, the abundant presence of non-pigmented tumor cells in the patient’s CSF and histopathologic exam might explain the mixed MRI signals. Some T1W hyperintense and T2W hypointense lesions were observed around the right splenial sulci, which corresponded to the densely pigmented and thickened meninges on gross pathology. Moreover, the multifocal hemorrhage observed on histopathologic examination in the present case might have changed the signal intensity variably based on the stage of the hemoglobin and the integrity of the red blood cell walls. On the other hand, imaging investigation in meningeal melanomatosis can also be very frustrating. One human case series described that brain MRI performed within the first month from disease onset could be normal despite the development of obvious neurologic signs. Indirect signs like dilation of the ventricular system and reduction of subarachnoid sulci should raise the clinician’s suspicion of a meningeal neoplasm [3].

In human medicine, meningeal melanomatosis, lymphomatosis, carcinomatosis, and gliomatosis are termed neoplastic meningitis, which consists of tumor cells diffusely infiltrating the subarachnoid space. This can be metastatic or a primary CNS originated neoplasm. Detection of malignant cells on cytologic examination of the CSF is the gold standard to make the diagnosis [10]. Three meningeal carcinomatosis and three gliomatosis cases have been documented in dogs, but CSF analysis was only performed in one dog and the results were unremarkable [4, 21–23, 26]. In one case series of canine and feline CNS lymphoma, abnormal meninges on MRI were seen in 10 out of 12 cases. The antemortem diagnosis was established through CSF cytologic evaluation in half of those cases [18]. Although diagnosis of primary CNS melanomas without biopsy is challenging, aggressive subarachnoid space invasion in meningeal melanomatosis may raise the odds of the presence of malignant cells in the CSF. In some human cases, as well as in our case, malignant melanocytes were observed in the CSF [3, 9, 15]. Although the probability of detecting malignant melanocytes in the CSF remains unclear in human literature, repeating CSF tap may be beneficial, since the first tap could only reveal elevated protein and pleocytosis without neoplastic cells, even when lesions were found on MRI [3, 9]. In a case series, two out of three patients had malignant melanocytes in the CSF, but in one of them, neoplastic cells were only detected from the repeated CSF tap [3]. Studies evaluating all types of neoplastic meningitis further support this concept. Malignant cells are detected in the CSF of 70–89% of patients, but only 50% of patients manifest positive CSF cytology by the initial lumbar puncture [11]. Another issue we encountered for this patient was the large number of undetermined round cells in CSF having no pigment granules. Most likely, these were plasma cells or amelanotic cells as observed on necropsy. However, round cells in CSF have also been found in other CNS neoplasms, including medulloblastomas, plasma cell tumors, metastatic mammary gland tumors, and choroid plexus papillomas [2, 12, 29, 30]. Another approach, such as the use of immunocytochemistry targeting Melan-A on the CSF sample, may clarify this and should be considered in the future. A similar technique has been utilized in human cutaneous amelanotic melanoma on fine-needle aspiration cytology [24].

In humans, no standard treatment has been established for primary CNS malignant melanoma due to its rarity. If the lesion is a focal mass lesion, a fair prognosis with the average survival of 7 years can be achieved after gross total resection [7]. However, in patients affected by meningeal melanomatosis, the prognosis becomes very poor. The overall median survival period is around 10 weeks after diagnosis, since these tumors are usually resistant to traditional radiotherapy and chemotherapy [13]. Fortunately, since understanding of the genetic and immunologic mechanisms underlying melanoma has increased recently, new drugs which target the pathology at a molecular level, such as the BRAF inhibitor vemurafenib and the anti-programmed cell death-1 antibody nivolumab have been developed and are reported to be effective against some malignant CNS melanomas [5, 27]. Besides targeted therapies, immunotherapy has evolved enormously in the last decade for both human and veterinary cancer management. Interest in the use of immunotherapy has grown based on some favorable outcomes in canine mucosal melanoma treated with checkpoint inhibitors and vaccines, although these still need large, randomized, prospective and double-blinded clinical trials [1, 25].

Meningeal melanomatosis is an extremely rare variant of CNS melanoma. Our case unveils the similarity of this tumor’s characteristics in humans and dogs. The characteristic T1W hyperintensity and strong contrast enhancement in our case were this disease’s most discriminative findings compared to other meningeal diseases. CSF analysis is still an invaluable tool for antemortem diagnosis in some cases.
CONFLICTS OF INTEREST. The authors declare that they have no conflicts of interest related to the subject materials discussed in this article.

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REFERENCES
1. Almela, R. M. and Ansón, A. 2019. A Review of immunotherapeutic strategies in canine malignant melanoma. *Vet. Sci*. 6: 15. [Medline] [CrossRef]
2. Behling-Kelly, E., Petersen, S., Muthuswamy, A., Webb, J. L. and Young, K. M. 2010. Neoplastic pleocytosis in a dog with metastatic mammary carcinoma and meningeal carcinomatosis. *Vet. Clin. Pathol.* 39: 247–252. [Medline] [CrossRef]
3. Berzner, G., Diamanti, L., Di Stefano, A. L., Bini, P., Franciotta, D., Iarossi, I., Pedrazzoli, P., Magrassi, L., Morfini, P., Farina, L. M., Bastianello, S., Ceroni, M. and Marchioni, E. 2015. Meningeal carcinomatosis: a challenge for timely diagnosis. *Biomed Res. Int.* 2015: 948497. [Medline] [CrossRef]
4. Canal, S., Bernardini, M., Pavone, S. and Mandara, M. T. 2013. Primary diffuse leptomeningeal gliomatosis in 2 dogs. *Can. Vet. J.* 54: 1075–1079. [Medline]
5. Chapman, P. B., Hauschild, A., Robert, C., Hainen, J. B., Ascierto, P., Larkin, J., Dummer, R., Garbe, C., Testori, A., Maio, M., Hogg, D., Lorigan, P., Lebbe, C., Jouary, T., Schadendorf, D., Ribas, A., O’Day, S. J., Sosman, J. A., Kirkwood, J. M., Eggermont, A. M. M., Dreno, B., Nolop, K., Li, J., Nelson, B., Hou, J., Lee, R. J., Fhlethy, K. T., McArthur G. A., BRIM-3 Study Group. 2011. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N. Engl. J. Med.* 364: 2507–2516. [Medline] [CrossRef]
6. Felix, C. C., Hyde, J. S., Sarna, T. and Sealy, R. C. 1978. Interactions of melanin with metal ions. Electron spin resonance evidence for chelate complexes of metal ions with free radicals. *J. Am. Chem. Soc.* 100: 3922–3926. [CrossRef]
7. Fujimori, K., Sakai, K., Higashiyama, F., Oya, F., Majeima, T. and Miyake, T. 2018. Primary central nervous system malignant melanoma with leptomeningeal carcinomatosis: a case report and review of the literature. *Neurosurv. Res.* 41: 333–339. [Medline] [CrossRef]
8. Galán, A., Martín-Suárez, E. M., Mollèda, J. M., Raya, A., Gómez-Laguna, J. and Martín De Las Mulas, J. 2009. Presumed primary uveal melanoma with brain extension in a dog. *J. Small Anim. Pract.* 50: 306–310. [Medline] [CrossRef]
9. Garbacz, T., Osuchowski, M. and Bartosik-Puszek, H. 2019. Primary diffuse meningeal carcinomatosis - a rare form of meningeal carcinoma: case report. *MBC Neuro. 19*: 271. [Medline] [CrossRef]
10. Glantz, M. J., Cole, B. F., Glantz, L. K., Cobb, J., Mills, P., Lekos, A., Walters, B. C. and Recht, L. D. 1998. Cerebrospinal fluid cytology in patients with cancer: minimizing false-negative results. *Cancer* 82: 733–739. [Medline] [CrossRef]
11. Gleissner, B. and Chamberlain, M. C. 2006. Neoplastic meningitis. *Lancet Neurol.* 5: 443–452. [Medline] [CrossRef]
12. Greenberg, M. J., Schatzberg, S. J., deLahunta, A., Bawn, T. and Summers, B. A. 2004. Intracerebral plasma cell tumor in a cat: a case report and literature review. *J. Vet. Intern. Med.* 18: 581–585. [Medline] [CrossRef]
13. Harstadt, L., Hess, K. R. and Groves, M. D. 2008. Prognostic factors and outcomes in patients with leptomeningeal carcinomatosis. *Neuro-oncol.* 10: 1010–1018. [Medline] [CrossRef]
14. Hayward, R. D. 1976. Malignant melanoma and the central nervous system. A guide for classification based on the clinical findings. *J. Neurol. Neurosurg. Psychiatry* 39: 526–530. [Medline] [CrossRef]
15. Kim, D. H., Choi, C. Y., Lee, C. H. and Joo, M. 2015. Primary intracranial leptomeningeal carcinomatosis. *J. Korean Neuurosurg. Soc.* 58: 554–556. [Medline] [CrossRef]
16. Kim, D. Y., Royal, A. B. and Villamil, J. A. 2009. Disseminated melanoma in a dog with involvement of leptomeninges and bone marrow. *Vet. Pathol.* 46: 80–83. [Medline] [CrossRef]
17. Kleinschmidt-DeMasters, B. K. and L. T. 2010. Infections and inflammatory disorders. pp. 455–484. In: Practical Surgical Neuropathology: A Diagnostic Approach (Perry, A. and Brat, D. J. eds.), Churchill Livingstone/Elsevier, Philadelphia.
18. LaRue, M. K., Taylor, A. R., Buc, A. R., Lindley, S. E., Boudreaux, B. L., Almond, G. T., Shores, A., Brawner, W. R. and Smith, A. N. 2018. Central nervous system lymphoma in 18 dogs (2001 to 2015). *J. Small Anim. Pract.* 59: 547–552. [Medline] [CrossRef]
19. Lee, P. H., Wang, L. C. and Lee, E. J. 2017. Primary intracranial melanoma. *J. Cancer Res. Pract.* 4: 23–26. [Medline] [CrossRef]
20. Liubinas, S. V., Maertens, N. and Drummond, K. J. 2010. Primary melanocytic neoplasms of the central nervous system. *J. Clin. Neuros.* 17: 1227–1223. [Medline] [CrossRef]
21. Lobacz, M. A., Serra, F., Hammond, G., Oevermann, A. and Haley, A. C. 2018. Imaging diagnosis - magnetic resonance imaging of diffuse leptomeningeal oligodendrogliomatosis in a dog with “dural tail sign”. *Vet. Radiol. Ultrasound* 59: E1–E6. [Medline] [CrossRef]
22. Mandara, M. T., Rossi, F., Lepri, E. and Angeli, G. 2007. Cerebellar leptomeningeal carcinomatosis in a dog. *J. Small Anim. Pract.* 48: 504–507. [Medline] [CrossRef]
23. Mateo, I., Lorenzo, V., Muñoz, A. and Molin, J. 2010. Meningeal carcinomatosis in a dog: magnetic resonance imaging features and pathological correlation. *J. Small Anim. Pract.* 51: 43–48. [Medline] [CrossRef]
24. Mondal, S. K., Mondal, P. K. and Dutta, S. K. 2014. Cytodiagnosis of epithelioid malignant melanoma (amelanotic) and diagnostic dilemmas. *J. Cytol.* 31: 207–209. [Medline] [CrossRef]
25. Prouteau, A. and André, C. 2019. Canine melanomas as models for human melanomas: clinical, histological, and genetic comparison. *Genes (Basel)* 10: 501. [Medline] [CrossRef]
26. Pumarola, M. and Balasch, M. 1996. Meningeal carcinomatosis in a dog. *Vet. Rec.* 138: 523–524. [Medline] [CrossRef]
27. Robert, C., Long, G. V., Brady, B., Dutriaux, C., Maio, M., Mortier, L., Hassel, J. C., Rutkowski, P., McNeil, C., Kalinka-Warzocha, E., Savage, K. J., Herrnberg, M. M., Lebbé, C., Charles, J., Mihaleciou, C., Chiarion-Sileni, V., Mauch, C., Cognetti, F., Arance, A., Schmidt, H., Schadendorf, D., Gogas, H., Lundgren-Eriksson, L., Horak, C., Sharkey, B., Waxman, I. M., Atkinson, V. and Ascierto, P. A. 2015. Nivolumab in previously untreated melanoma without BRAF mutation. *N. Engl. J. Med.* 372: 2507–2516. [Medline] [CrossRef]
28. Smith, A. B., Rushing, E. J. and Smirniotopoulos, J. G. 2009. Pigmented lesions of the central nervous system: radiologic-pathologic correlation. *Radiographics* 29: 1503–1524. [Medline] [CrossRef]
29. Thompson, C. A., Russell, K. E., Levine, J. M. and Weeks, B. R. 2003. Cerebrospinal fluid from a dog with neurologic collapse. *Vet. Clin. Pathol.* 32: 143–146. [Medline] [CrossRef]
30. Westworth, D. R., Dickinson, P. J., Vernau, W., Johnson, E. G., Bollen, A. W., Kass, P. H., Sturges, B. K., Vernau, K. M., Lecouteur, R. A. and Higgins, R. J. 2008. Choroidal nevus tumours in 56 dogs (1985–2007). *J. Vet. Intern. Med.* 22: 1157–1165. [Medline] [CrossRef]