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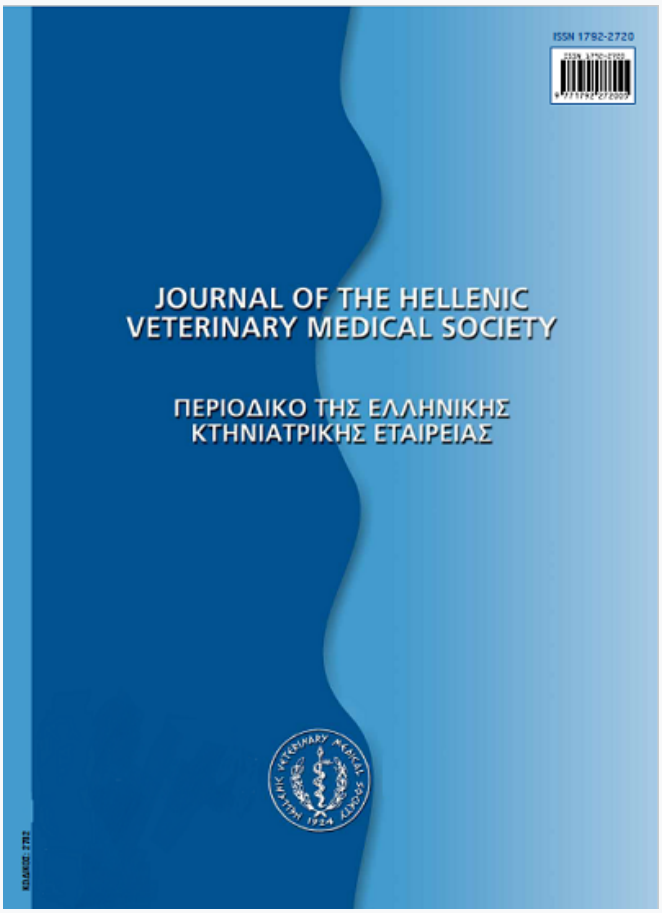

Rhabdomyosarcomas arising from striated muscles in elderly dogs: pathological features of 4 cases

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Rhabdomyosarcomas arising from striated muscles in elderly dogs: pathological features of 4 cases

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Ραβδομυοσάρκωμα των γραμμωτών μυών σε παρήλικες σκύλους: παθολογοανατομική μελέτη 4 περιστατικών

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ABSTRACT. Primary rhabdomyosarcomas are rare in dogs. Based on their classification, embryonal rhabdomyosarcoma is the most common, while alveolar and especially pleomorphic types occur less often. Four cases diagnosed as primary canine rhabdomyosarcomas of striated muscles were retrieved from our files. All the animals were cross-breeds, aged over 8 years. Two of them had died after developing disseminated intravascular coagulation and gastric ulcer, respectively, and two others were euthanized. Of those two, one had been admitted with neurological and cardiovascular symptoms and one with disseminated intravascular coagulation. Necropsy was performed and tissue samples were collected for histological and immunohistochemical examination. The first case was diagnosed as mixed rhabdomyosarcoma, pleomorphic type in the heart and the diaphragm and alveolar type in the lungs and the spleen. The three other cases were of alveolar type. One showed primary cardiac and oesophageal origin, with metastases in the skeletal muscles and non-striated muscle tissues, one had primary cardiac, with mitral valve involvement, and skeletal muscle origin, with metastases in extra-striated muscle tissues and one showed only skeletal muscle localization. Immunohistochemical examination revealed myoglobin and α-sarcomeric actin in tumour cells.

Keywords: alveolar, dog, immunohistochemistry, pleomorphic, rhabdomyosarcoma

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ΠΕΡΙΛΗΨΗ. Τα πρωτογενή ραβδομυοσαρκώματα απαντώνται σπάνια στους σκύλους. Ανάλογα με την ταξινόμησή τους, διακρίνονται ως εμβρυϊκά που αποτελούν το συνηθέστερο τύπο ραβδομυοσαρκώματος, κυψελιδικά και πλειομορφικά. Τα δύο τελευταία, ιδιαίτερα τα πλειομορφικά, έχουν αναφερθεί σπάνια σε σκύλους. Στην παρούσα μελέτη περιγράφονται τέσσερα περιστατικά από το αρχείο μας με διάγνωση πρωτογενούς ραβδομυοσαρκώματος στους γραμμωτούς μυς σκύλων. Όλα τα ζώα ήταν ημίαιμα, ηλικίας μεγαλύτερης των 8 ετών. Στο πρώτο ζώο, ο θάνατος επήλθε μετά από εκδήλωση διάσπαρτης ενδοαγγειακής πήξης και στο δεύτερο ζώο μετά από εμφάνιση γαστρικού έλκους. Αντίθετα, στο τρίτο και το τέταρτο διενεργήθηκε ευθανασία μετά από συνεννόηση με τους ιδιοκτήτες. Το τρίτο ζώο προσκομίστηκε με συμπτώματα από το νευρικό και το καρδιοαγγειακό σύστημα και το τέταρτο ζώο με διάσπαρτη ενδοαγγειακή πήξη. Αμέσως μετά το θάνατό τους, τα ζώα νεκροτομήθηκαν και λήφθηκαν ιστοτεμάχια για ιστοπαθολογική και ανοσοϊστοχημική διερεύνηση. Στον πρώτο σκύλο, τέθηκε διάγνωση μικτού ραβδομυοσαρκώματος, με τον πλειομορφικό τύπο να εντοπίζεται στην καρδιά και το διάφραγμα και τον κυψελιδικό στους πνεύμονες και το σπλήνα. Στους υπόλοιπους τρεις σκύλους, διαπιστώθηκε ο κυψελιδικός τύπος ραβδομυοσαρκώματος. Στο ένα ζώο εντοπίστηκαν πρωτογενείς εστίες στην καρδιά και τον οισοφάγο, καθώς και μεταστατικές εστίες στους σκελετικούς μυς και σε όργανα που φυσιολογικά δεν φέρουν γραμμωτό μυϊκό ιστό. Το τρίτο ζώο εμφάνιζε πρωτογενή εντόπιση στο μυοκάρδιο, με προσβολή και της μιτροειδούς βαλβίδας, καθώς και στους σκελετικούς μυς. Στο ζώο αυτό, παρατηρήθηκαν μεταστάσεις σε όργανα που φυσιολογικά δεν φέρουν γραμμωτό μυϊκό ιστό. Τέλος, στο τέταρτο ζώο, το νεόπλασμα περιοριζόταν μόνο στους σκελετικούς μυς. Η διάγνωση επιβεβαιώθηκε ανοσοϊστοχημικά, με τη θετική αντίδραση των νεοπλασματικών κυττάρων στην παρουσία μυογλοβίνης και σαρκομερικής-α ακτίνης. Ιδιαίτερη σημασία παρουσιάζει η εντόπιση του νεοπλάσματος στο μυοκάρδιο τριών ζώων. Επίσης, για πρώτη φορά αναφέρεται η παρουσία του πλειομορφικού ραβδομυοσαρκώματος στο διάφραγμα και του κυψελιδικού ραβδομυοσαρκώματος σε γραμμωτό μυ, σε σκύλους ηλικίας άνω των 8 ετών.

Λέξεις ευρετηρίασης: ανοσοϊστοχημεία, κυψελιδικό, πλειομορφικό, ραβδομυοσάρκωμα, σκύλος

INTRODUCTION

Rhabdomyosarcomas (RMSs) are malignant neoplasms of striated muscles, which originate from muscle progenitor mesenchymal cells or from myocytes undergoing neoplastic transformation (Gonin-Jmaa et al., 1996). Sporadic cases of RMS have been reported in animals, including dogs (Worley and Gorham, 1954; Krotje et al., 1990; Gonin-Jmaa et al., 1996; Pérez et al., 1998; Cooper and Valentine, 2002; Ginel, 2002; Chijiwa et al., 2004; Aupperle et al., 2007; Yhee et al., 2008), cats (Mincus and Hillemanns, 1997), horses (Clegg and Coumbe, 1993) and sheep (Yener, 2001). Following the respective tumour classification methodology in humans, RMSs in domestic animals are classified based on histopathological findings as embryonal, botryoid (variant of embryonal), alveolar and pleomorphic RMS (Cooper and Valentine, 2002).

In humans, RMS is the most prevalent soft tissue sarcoma of children and adolescent individuals. In people younger than 20 years, the most often type found is the embryonal, followed by the botryoid and the alveolar. Although RMSs may occur in any anatomical site of young individuals, a predilection for the head and neck, genitourinary areas, retroperitoneum and extremities has been recorded. Of note is the frequently observed occurrence of primary RMSs in tissues containing little or no skeletal muscle. In adults though, RMS do not occur that often. The most prevalent type in that age group is the pleomorphic RMS and the most common site of origin is the skeletal muscles of the limbs (Cooper and Valentine, 2002; Yasuda et al., 2009).

In dogs, a pattern of localisation for RMS has not yet been recognised. However, there seems to be a trend of similarity with what has been reported in humans, regarding the fact that the majority of RMSs in dogs have occurred in tissues that normally do not contain striated-muscle cells, such as the pharynx, gingiva, urethra, trachea, larynx and the jawbone (Seibold, 1974; Sarnelli et al.,1994; Yanoff et al., 1996; Ginel et al., 2002;, Illanes, 2002; Kobayashi et al., 2004; Suzuki et al., 2006; Bae et al., 2007; Murakami et al., 2010). A few cases of canine RMSs have been reported to arise from striated muscles (Worley and Gorham, 1954; Gonin-Jmaa et al., 1996; Kim et al., 1996; Lascelles et al., 1998; Pérez et al., 1998; Cooper and Valentine, 2002; Machida et al., 2003; Brockus and Myers, 2004; Akkoc et al., 2006; Aupperle et al., 2007; Nakaichi et al., 2007; Chapman et al., 2008; Yhee et al., 2008; Yamate et al., 2011) and only some of them were further classified. Most of these RMSs were of the embryonal type. Three of them were found to be locat-

ed in the head and three in large skeletal muscles in the flank and axilla (Kim et al., 1996; Lascelles et al., 1998; Cooper and Valentine, 2002; Yhee et al., 2008). Four tumours were diagnosed as pleomorphic and originated from the neck muscles, the tongue and the heart (Pérez et al., 1998; Cooper and Valentine, 2002; Chapman et al., 2008; Yamate et al., 2011). The alveolar type, on the other hand, has been reported thusfar in five dogs, with a common characteristic among them being the extra-striated muscle origin. Specifically, alveolar RMS was found in the gingiva, the orbit and the genital tract of ftyfour young dogs and in the urinary bladder of a 9-year old dog (Seibold, 1974; Sarnelli et al., 1994; Kim et al., 1996; Bae et al., 2007; Murakami et al., 2010).

Further reports of canine RMS can help to reveal a pattern of breed, age, gender and anatomical site prevalence for each one of the different histopathological types of this relatively rare tumour in the dog. Herebelow, we report four unusual cases of RMS, observed in old dogs. Three cases were classified as alveolar RMSs. One of these originated from the heart, the oesophagus and the skeletal muscles, another from the heart and the skeletal muscles and the third from the skeletal muscles. The remaining case was mixed: pleomorphic RMS in the cardiac muscle and the diaphragm and alveolar RMS in the lungs and the spleen.

To the best of our knowledge, these are the first reports of classic alveolar RMS in skeletal muscle, cardiac muscle and oesophagus of dogs and, also, the first report of skeletal muscle RMS in adult dogs resembling juvenile alveolar variant of RMS reported previously in dogs and humans. In addition, this report includes the first case of diaphragmatic pleomorphic RMS in dogs.

MATERIALS AND METHODS

Four old (age range: 8-14 years) dogs with RMS were submitted to the Department of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki. Necropsy was performed and tissue samples were collected, fixed in 10% neutral buffered formalin, embedded in paraffin wax and cut in 5 μm thick sections for haematoxylin and eosin (H&E) and phosphotungstic acid haematoxylin (PTAH) stains. Serial sections from the same samples were used on positively charged slides for immunohistochemical detection of α-smooth muscle actin (smA, clone 1A4), α-sarcomeric actin (scrA, clone alpha-Sr-1), vimentin (clone V9), cytokeratin (clone AE1/AE3) (Dako; Denmark) and myoglobin (Mb, rabbit polyclonal) (Spring; USA) using the EnVision method. After deparafinization and rehydration, the sections were boiled in an autoclave in EDTA buffer for antigen unmasking and, then, immersed in H2O2, 3% in phosphate buffered saline (PBS) for blocking endogenous peroxidase; this was followed by incubation with the primary antibodies diluted in phosphate buffered saline (PBS). Subsequently, incubation of sections with peroxidase labeled polymer (EnVision+ System-HRP, goat anti-rabbit IgG and goat anti-mouse IgG - for polyclonal and monoclonal antibodies, respectively; Dako; Denmark) was performed and 3, 3 diaminobenzidine (DAB) was used as chromogen for signal detection. The slides were counterstained with Haematoxylin Mayer`s. Negative controls were performed by omitting the primary antibodies. Normal striated muscle cells were used as internal positive controls for Mb and scrA, smooth muscle cells of the vessels for smA, connective tissue fibroblasts for vimentin and oesophageal epithelial cells for cytokeratin.

RESULTS

At necropsy, in case 1 (9-year old), we found whitish to grey, mostly large rod-like and diffusely arranged areas in the myocardium of the right and left ventricle. The whole left and right atrial myocardium was solid and rigid and showed thickening and grayish discoloration (Fig. 1). Whitish regions were also observed in the diaphragm. Microscopically, H&E stained sections of the heart and the diaphragm revealed presence of neoplastic foci, varying in size, comprising mostly of haphazardly arranged spindle and angular cells and, to a lesser extent, of ovoid cells, with cystic round to ovoid nuclei and prominent nucleoli, characteristic of pleomorphic RMS (Fig. 2). Atypical mitoses were a commonly seen (2-3 per high power field). Cross

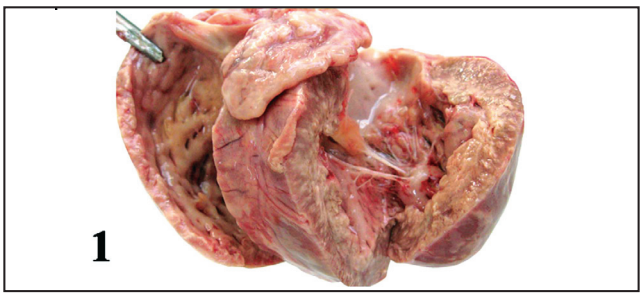

Fig. 1. Dog No. 1, heart. Pleomorphic RMS: multiple, white to grey, firm neoplastic foci infiltrating approximately the whole heart.

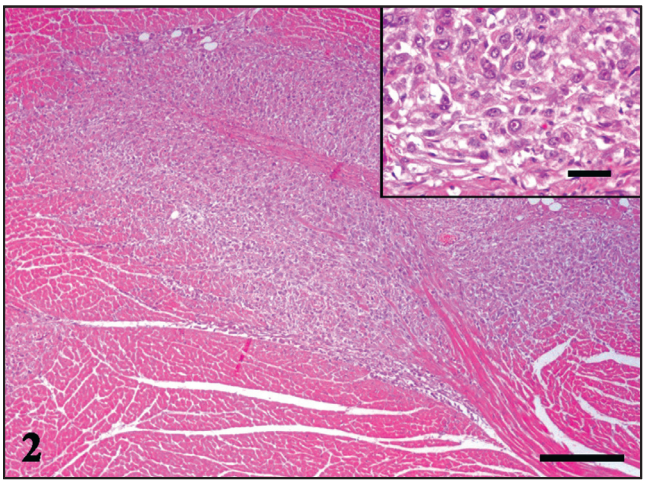

Fig. 2. Dog No. 1, heart mass. Pleomorphic RMS: neoplastic cells effacing the normal myocardium (bar =100 μm). Inset: spindle, strap-like and ovoid cells with eosinophilic cytoplasm, haphazardly arranged, containing round to ovoid nucleus with a prominent nucleolus (bar = 25 μm).

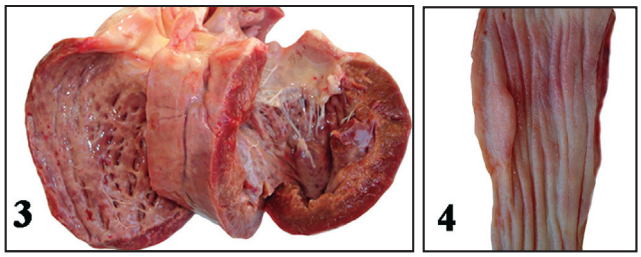

Fig. 3. Dog No. 2, heart. Alveolar RMS: grey to yellowish areas diffusely arranged, occupying mostly the subendocardial myocardium.

Fig. 4. Dog No. 2, oesophagus. Alveolar RMS: diffuse thickening of the esophagus wall and an ovoid mass projecting into its lumen.

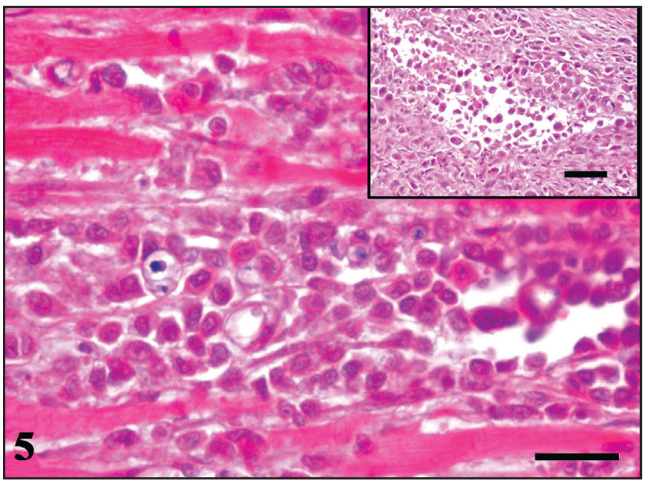

Fig. 5. Dog No. 2, heart mass. Alveolar RMS: aggregates of small neoplastic cells with eosinophilic or vacuolated cytoplasm among cardiac muscle cells (bar =25 μm). Inset: area with alveolar pattern (bar = 50 μm).

striation was seen in the cytoplasm of a few angular or strap like neoplastic cells, using the PTAH staining. Tumour cells were shown in hepatic and pulmonary vessels. Additionally, rhabdomyosarcomatous foci of the alveolar type were demonstrated in tissues that, normally, did not contain striated muscle cells, such as the lungs and the spleen.

In case 2 (11-year old), yellowish to gray areas were present, distributed diffusely in the myocardium of both ventricles and atria (Fig. 3). In the upper part of the oesophagus, a mucosa covered mass, 3-cm in diametre, protruding into the lumen, and whitish discoloration of the muscles of the lower 3rd of this organ, were obvious (Fig. 4). The mediastinal lymph-nodes were intumesced and tan in colour, whilst grey firm patches where seen in the lungs. The neoplastic foci of the heart and the oesophagus shared similar histological features. The majority of neoplastic cells were round or ovoid in shape with large round to oval nuclei and scant to clear vacuolated cytoplasm. Mitotic figures were approximately 1 per high power field. Alveolar-like formations were rarely seen and were lined by small poorly differentiated cells and large cells with eosinophilic cytoplasm and eccentrically located nucleus. The alveolar lumens were either clear or occupied by the same type of cells some of which were degenerated, as described in classical alveolar RMS, but dense fibrous tissue surrounding the alveoli was lacking (Fig. 5). Metastases were found in skeletal muscle, lung, lymph node and intestinal subserosal adipose tissue sections. Neoplastic cells were detected within hepatic vessels.

In case 3 (14-year old), the left hind limb was intumesced with pale skeletal muscle discoloration. A large-sized encapsulated abscess-like mass in the quadriceps femoris muscle and smaller masses in muscles of the lumbar area were detected. Haemopericardium, distention of the left ventricle and presence of cauliflower-like growth on the mitral valve were detectable. In the lungs, numerous nodules of small diameter (~0.5 cm) with the appearance of infarcts or small abscesses were observed. Multiple nodules were also seen in the kidneys, the intestine and the brain cortex. Histological examination revealed lesions in the heart and the skeletal muscles, consisting mostly of multiple nests small in size and separated by thin fibrous connective tissue admixed with spindle cells. These vague alveoli showed close resemblance to those detected in case

No. 2. Mitoses were seen in 2 neoplastic cells per high power field. No cell remnants were observed in the centre of the above lesions (Fig. 6). Alveolar RMS was also detected in lung, kidney, liver, adrenal gland, intestinal muscle and brain sections of this case.

In case 4 (8-year old), the left fore and hind limbs were oedematous and firm subcutaneous intumescences were detected in the perineal and the glouteal area, as well as in the mammary glands of the left side. The cut surfaces of the muscles of the above regions were primarily firm and gray-white in colour. Histologically, characteristic lesions of the classic alveolar type were prominent. These lesions were composed of cells forming circular, alveolar-like structures containing within their lumen, small neoplastic round or ovoid cells admixed with larger cells containing eosinophilic cytoplasm and one to two hyperchromatic, eccentric nuclei. The majority of the large cells were adherent to the fibrous septa. Most of the cells aggregated in the centre of the lumen were degenerating, accompanied by necrotic debris. Round areas with alveolar pattern, numerous neoplastic spindle and ovoid cells lying within the fibrous stroma, were prominent (Fig. 7). Mitoses were rarely seen (1 per high power field). No histological changes were detected in heart sections. Tumour cells were also found within hepatic, splenic and renal vessels. In a kidney, an infarcted area was present, probably secondary to vascular occlusion by neoplastic cell emboli.

Focal necrosis within the neoplastic areas was recorded in all 4 animals examined. Immunohistochemical examination revealed strong positivity for most of the neoplastic cells in case 1 (Fig. 8) and weak to moderate in cases 2-4, using the antibodies against scrA. In contrast, the staining intensity of Mb was more remarkable in cases 2-4 than in case 1 (Fig. 9). A small number of tumour cells showed strong positivity for cytokeratin in case 2, while no immunoreactivity was detected with asmA or vimentin in the neoplastic cells.

General details regarding the four cases are presented in Table 1. The immunohistochemical findings using the antibodies against scrA and Mb are summarized in Table 2.

DISCUSSION

We describe four cases of canine RMS dealt with over a 6-year period. One of the cases was diagnosed

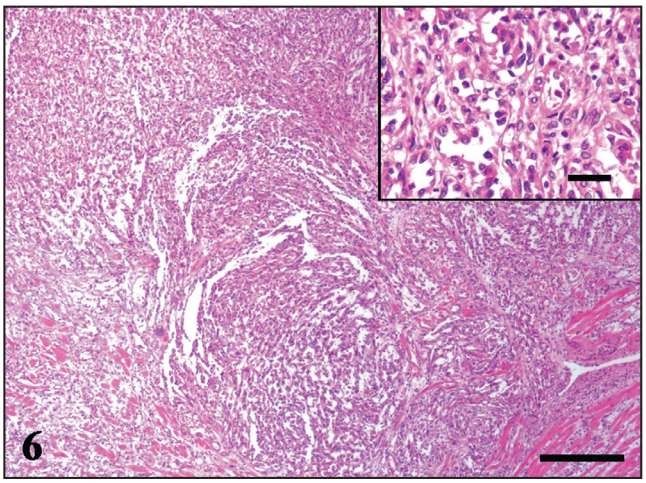

Fig. 6. Dog No. 3,skeletal muscle mass. Alveolar RMS: a large region occupied by tumour cells forming small alveoli. (bar =100 μm). Inset: higher magnification from the previous figure (bar = 25μm).

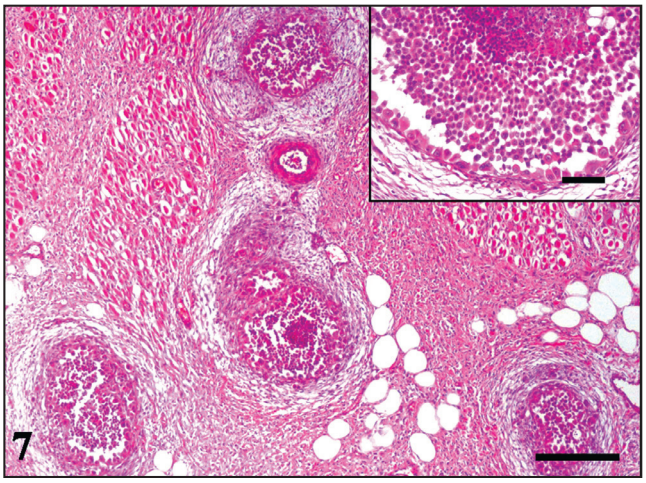

Fig. 7. Dog No. 4, skeletal muscle mass. Alveolar RMS: four foci with the classic alveolar pattern (bar =100 μm). Inset: large uni-or binucleated cells with eosinophilic cytoplasm lining the fibrous septa and others 'floating' into the lumen most of which are degenerating (bar = 50 μm).

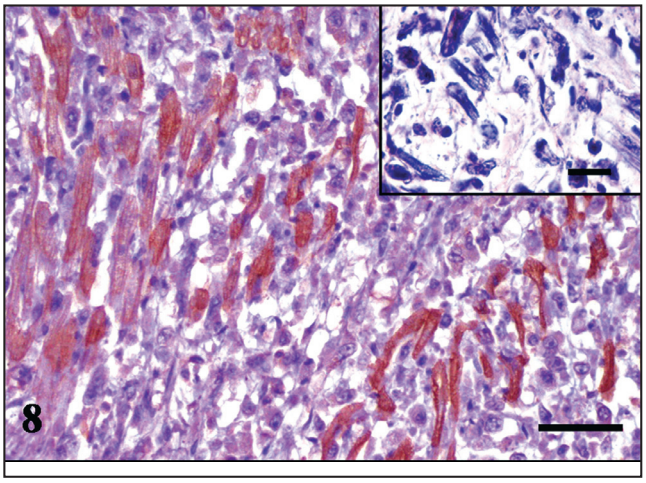

Fig. 8. Dog No. 1, heart mass. Elongated neoplastic cells, intensely positive and ovoid cells, weakly positive for scrA (EnVision, HRP, Harris's hematoxylin counterstain, bar = 50 μm). Inset: racket cells with cross striations (PTAH staining, bar =25 μm).

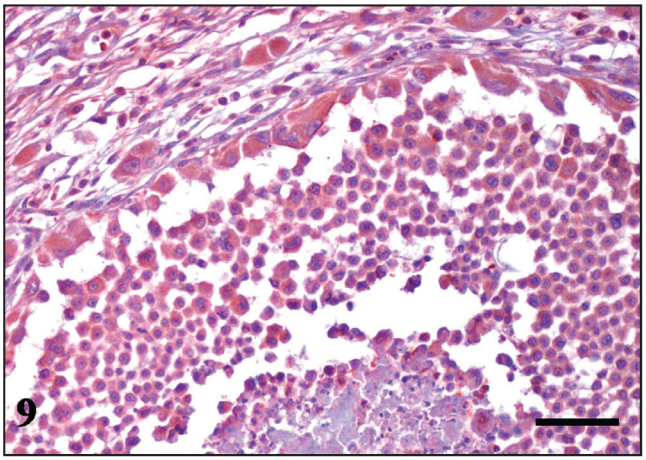

Fig. 9. Dog No. 4, skeletal muscle mass. Numerous cells showing strong cytoplasmic positivity for Mb (EnVision, HRP, Harris's hematoxylin counterstain, bar = 50 μm).

as mixed: pleomorphic in striated muscles and alveolar in the lungs and the spleen (Case 1). It is noteworthy that the pleomorphic type is extremely rare and, in adult humans, it is usually seen in large muscles of the limbs. In the present case, the tumour was found in the heart and the diaphragm. To date only 4 canine pleomorphic RMSs have been described: two involving the muscles of the neck, one involving the tongue and one involving exclusively the heart (Cooper and Valentine, 2002; Pérez et al., 1998; Chapman et al., 2008; Yamate et al., 2011).

The histological pattern of RMS in cases 2 and 3 was similar to that usually observed in previous reports of alveolar RMS in dogs and humans, where irregular masses of neoplastic cells or vague alveolar formations predominate (Seibold, 1974; Yasuda et al., 2009). In case 2, metastatic alveolar RMS was found and involved the heart, the oesophagus, skeletal muscles, the lungs, the adipose tissue and lymph nodes. Neoplastic cells with vacuolated to clear cytoplasm were a characteristic finding in this case. In human cases of alveolar RMS, this finding has been reported as a rare histologic feature, the presence of which may complicate diagnosis (Yasuda et al., 2009). Cytoplasmic vacuolation has been previously reported in a dog with cardiac pleomorphic RMS (Pérez et al., 1998), but the large number of such cells observed in the present case, as well as the histopathological and immunohistochemical features that characterize alveolar RMS were compatible with those described in the gingiva of a 11-month-old dog and in humans (Seibold, 1974; Cooper and Valentine, 2002). Case 3 was diagnosed as alveolar RMS in striated muscles (cardiac-skeletal) with metastases in the lungs, the kidneys, intestinal muscles, the liver, the adrenal glands and the brain.

Non-metastatic alveolar RMS with its classic histological pattern demonstrated in humans was observed in skeletal muscles of case 4 (Cooper and Valentine, 2002). To our knowledge, similar lesions have never been reported in canine striated muscle tissues, but were described previously only in one dog with alveolar RMS located in the greater omentum (Sarnelli et al., 1994).

The tumours examined were considered to be non-congenital, since they occurred in old animals and were of striated muscle origin (Pérez et al., 1998). Moreover, the morphological and histological features were not similar to those described in round cell or myotubular type of embryonal RMS. Although alveolar RMSs are usually seen in young animals and humans and can occur in tissues other than striated muscles (Seibold,

Table 1. General details about four dogs with rhabdomyosarcoma and details of the tumour (P: pleomorphic, A: alveolar) location.

Case no.	Age	Breed	Gender	Cause / type of death	Primary tumour location	Secondary tumour location	Vascular infiltration
1	9	mixed	♀	Disseminated intravascular coagulation / Death	Heart & diaphragm(P) , lung & spleen (A)	-	Lungs, liver
2	11	mixed	♂	Regurgitation gastric ulcer / Post-operative death	Heart & oesophagus (A)	Skeletal muscles. lungs lymph nodes, adipose tissue	Liver
3	14	mixed	♂	Supraventricular extrasystoles, severe seizures / Euthanasia	Heart/mitral valve & skeletal muscles (A)	Lungs, kidneys, brain, intestine adrenal glands, liver	-
4	8	mixed	♀	Disseminated intravascular coagulation / Euthanasia	Skeletal muscles (A)	-	Kidneys, spleen, liver

Table 2. Immunohistochemical results of α-sarcomeric actin and myoglobin labeling in four dogs with rhabdomyosarcoma.

	Case no.							
	1		2		3		4	
	scrA	Mb	scrA	Mb	scrA	Mb	scrA	Mb
Heart	++	+	+	++	+	++	-	-
Skeletal muscles / diaphragm	++	+	+	++	+	++	+	++
Lungs	++/vl	+/vl	+	++	+	++	-	-
Kidney	-	-	-	-	+	+	vl	vl
Spleen	+	+	N/A	N/A	-	-	vl	vl
Lymph nodes nodes	N/A	N/A	+	+	N/A	N/A	N/A	N/A
Intestine	N/A	N/A	+	+	+	++	-	-
Adrenal glands	N/A	N/A	-	-	+	++	-	-
Brain	N/A	N/A	N/A	N/A	+	++	N/A	N/A
Liver	vl	vl	vl	vl	+	+	vl	vl

++: diffuse or strongly positive stain, +: focal or weak positive stain, -: no stain (negative result), vl: tumour cell immunopositivity within a vessel lumen, N/A: not available.

1974; Kim et al., 1996; Cooper and Valentine, 2002; Bae et al., 2007; Murakami et al., 2010), cases 2, 3 and 4 were 11, 14 and 8 years old respectively and the neoplasms originated from striated muscles. Besides, in previous studies, alveolar RMS was found in the bladder of a 9-year old dog and in the head and neck of 4 humans aged between 61 and 76 years (Sarnelli et al., 1994; Cooper and Valentine, 2002). This evidence led us to suggest that alveolar subtype may not be related exclusively to juvenile RMSs in dogs.

Difficulty in finding cells with cross striations has been stated by several authors in pleomorphic and mostly in alveolar RMS cases, even when using special methods like PTAH staining (Seibold, 1974; Pérez et al., 1998; Cooper and Valentine, 2002; Chapman et al., 2008). Accordingly, in our study cross striation was observed only in certain cells in case 1, in which pleopmorphic RMS was diagnosed.

There have been previous reports documenting the occurrence of canine RMS metastases (Seibold, 1974; Cooper and Valentine, 2002; Ginel et al., 2002; Akkoc et al., 2006; Yhee et al., 2008). In our study, metastases were found in cases 2 and 3, but no association was found between presence of metastases and malignancy grade. Much attention has been focused on the route of metastasis. The evidence of neoplastic cells within the vessels' lumen in organs with neoplasia or in the absence of neoplastic foci in all the cases examined is in concordance with previous studies on canine RMS, suggesting that metastases occurred through the haematogenous route. This has been stated in two reports of embryonal metastasizing RMS and one of cardiac metastasizing RMS (Kim et al., 1996; Akkoc et al., 2006; Yhee et al., 2008), but not in alveolar RMS as found in the present study.

Interestingly, the concomitant presence of two distinct variants (alveolar and pleomorphic RMS) found in different organs of the same animal (Case 1) reinforces the hypothesis of other investigators, which suggest that pleuripotential mesenchymal cells are simultaneously transformed into two different subtypes of RMS in their respective locations (Kim et al., 1996; Bae et al., 2007).

In old dogs RMSs that arise in sites that normally have striated muscle cells, are extremely rare. Immunohistochemical analysis was performed with several antibodies, including anti-myoglobin and anti-α sarcomeric actin. These markers are specific for striated muscle differentiation and for the diagnosis of rhabdomyosarcomas (Cooper and Valentine, 2002). The Mb and scrA positive immunostaining demonstrated in all dogs, confirmed the diagnosis of RMS, but their classification was performed based on the histological pattern of each case. Vimentin was not expressed in any of the 4 cases, since this protein is usually present only in small, poorly differentiated RMSs (Cooper and Valentine, 2002)

Although primary and secondary tumours involving the heart are uncommon in dogs, in our study, three out of four RMSs had cardiac localization. In humans, molecular characterization has proved useful for classification of RMS, when the histological features were not sufficient (Yasuda et al., 2009). A similar approach should be used in animal RMSs, when no definitive diagnosis can be established. To our knowledge, typical and non-typical striated muscle alveolar RMS and diaphragmatic pleomorphic RMS in dogs older than 8 years, are documented for the first time in the veterinary literature.

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