Pleural mesothelioma in a nine-month-old dog

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This paper reports on an unusual case of pleural epitheloid mesothelioma in a nine-month-old male, mixed breed dog. The dog was presented *in-extremis* and, on post mortem examination, multiple, exophytic, frequently pedunculated, yellowish-red, soft to firm masses ranging from 3mm to 6cm in diameter were diffusely distributed over, and attached to, the pericardial and parietal pleural surfaces. Microscopically, these masses consisted of round to partially polygonal-shaped, anaplastic cells with minimal cytoplasm and hyperchromatic nuclei covering papillomatous projections or as part of more densely cellular masses. A supporting fibrovascular stroma and mitotic figures were also evident. Constituent tumour cells were labeled positively with antibodies against both vimentin and cytokeratin. In contrast, the same cells exhibited equivocal labeling with an antibody directed against calretinin antigen and did not label with antibodies against carcinoembryonic antigen (CEA) and milk fat globule-related antigen (MFGRA). Such tumours are rare in dogs, particularly in such a young animal.

Key words: Mesothelioma, Pleura, Dog

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Introduction

Mesotheliomas are tumours of low grade malignancy originating from mesothelial cells covering the coelomic cavities such as the pericardium, pleura, peritoneum and vaginal tunic. These tumours can have a localised to a more diffuse distribution and may present as multiple to coalescing nodular, and sessile to more pedunculated, structures (Barker, 1993; Head *et al.*, 2002). Factors implicated in the pathogenesis of this neoplasm include exposure to dusts such as those of asbestos, iron, or silica (Barker, 1993; Cicala *et al.*, 1993; Head *et al.*, 2002), in addition to viral or genetic factors (Chabot *et al.*, 1970; Cacciotti *et al.*, 2001; Head *et al.*, 2002). In domestic animals, mesotheliomas are most frequently encountered in cattle and dogs, and occur most frequently as a congenital neoplasm in foetal or young cattle (Shin and Firminger, 1973; Goelz *et al.*, 1993; Webster *et al.*, 1993; Cunningham and Dhillon, 1998; Head *et al.*, 2002; Yener *et al.*, 2002). In species other than cattle, the occurrence of this neoplasm positively correlates with age so that it is rarely described in young animals (Barker, 1993; Head *et al.*, 2002; Kim *et al.*, 2002). In consequence, the case reported here, which involves a nine-month-old dog, is very unusual and merits documentation.

Case report

A nine-month-old male, mixed breed dog was presented to the clinic of the Ankara University Faculty of Veterinary Medicine *in-extremis* and died before clinical examination could be performed. A clinical history provided by the owner indicated that the animal had been dull, weak and dyspnoeic for the previous week. At necropsy, approximately 200 ml of serosanguinous fluid containing yellowish-red masses up to 2cm in diameter were observed in the thoracic cavity. Multiple to coalescing, exophytic, frequently pedunculated, yellowish-red, soft to firm masses ranging from 3mm to 6cm in diameter were diffusely

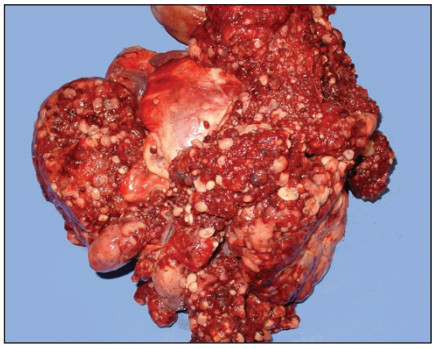

Figure 1: Diffuse distributed nodular, frequently pedunculated exophytic masses on pericardial and pleural surfaces.

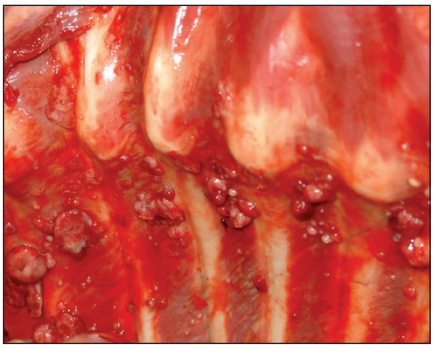

Figure 2: Multifocal exophytic masses on parietal pleura adjacent to costochondral junctions.

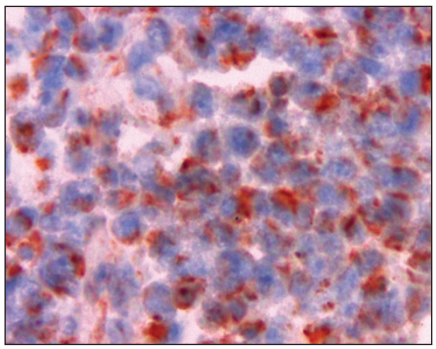

Figure 4: On immunohistochemistry, abundant cytoplasmic red-brown staining was observed in neoplastic cells using an antibody directed against vimentin. (ABC-P; original magnification x400).

distributed over, and attached to, the pericardial and parietal pleural surfaces (**Figures 1** and **2**). On cut section, clear fluid was noted within a number of the larger exophytic masses. The mediastinal lymph nodes were enlarged and, on cut-section, had wet surfaces. No other significant lesions were observed in the carcass.

Samples of the lesions were fixed in 10% buffered formalin, and were processed for histological examination by routine methods and embedded in paraffin wax. The sections were cut 5µm in thickness, mounted on glass slides and stained using the haematoxylin and eosin (HE) method. Further sections were dewaxed and rehydrated by routine methods for immunohistochemical staining as follows. Endogenous peroxidase activity in tissue sections was blocked by applying 0.3% hydrogen peroxide in methanol for 20 minutes, followed by treatment with pronase for 10 min at 40°C and incubation with normal goat serum for 20 min at 40°C. Test sections were then incubated for one hour at 40°C with each of the following monoclonal antibodies (all obtained from Dako/Denmark and all used at a 1:500 dilution) against vimentin: cytokeratin; carcinoembryonic antigen (CEA); calretinin; and, milk fat globule-related antigen (MFGRA). Sequential incubation with biotinylated goat anti-rabbit IgG and streptavidin-peroxidase reagent (Dako/Denmark) was then carried out. Colour labelling was developed by a final incubation step using 3-amino-9-ethyl-carbazole (AEC, Dako/Denmark) for five minutes at room temperature and sections were then counterstained with haematoxylin. Following each incubation step, sections were thoroughly washed with phosphate buffered saline (PBS) solution, except the step using normal goat sera. As a control step, sections were treated as above replacing the various primary antibodies with normal rabbit sera.

Microscopically, the lesions consisted of papillomatous projections to more solid nests of cells supported by a fine fibrovascular stroma. The constituent cells were round to polygonal in outline with minimal eosinophilic

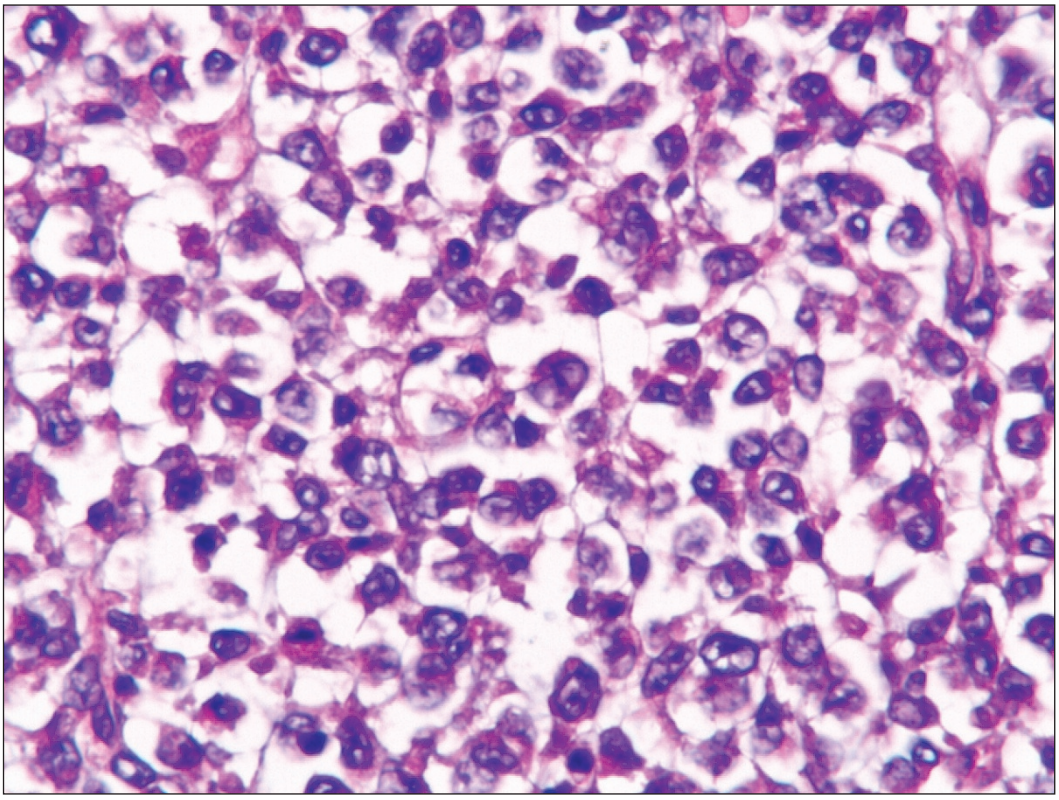

Figure 3: Clusters of round to polygonal cells with minimal eosinophilic cytoplasm and ovoid to angulated vesicular nuclei. (Haematoxylin and Eosin; original magnification x400).

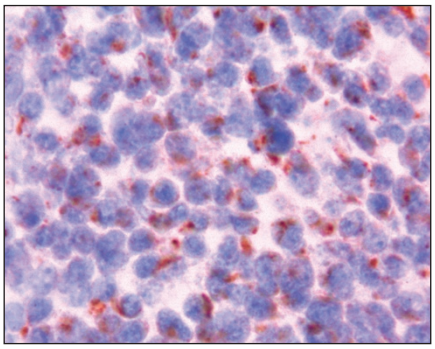

Figure 5: On immunohistochemistry, subtle red-brown cytoplasmic staining of neoplastic cells was observed using an antibody directed against cytokeratin. (ABC-P; original magnification x400).

cytoplasm and ovoid, to angulated vesicular nuclei (**Figure 3**). Anisokaryosis was a feature and mitotic figures were observed. Varying degrees of necrosis, inflammation and haemorrhage were noted within the masses. While there was evidence of lymphadenitis in the mediastinal lymph nodes, no evidence of neoplasia was observed. Immunohistochemically the tumour cells labelled positively with antibodies against vimentin (**Figure 4**) and cytokeratin (**Figure 5**). Inconclusive staining was obtained using calretinin antibody and no labelling was observed with the CEA and MFGRA antibodies.

Discussion

The gross and microscopic appearance of this neoplasm, together with its histochemical and immunohistochemical staining characteristics, are consistent with a diagnosis of epitheloid-type mesothelioma. These are primary tumours of low grade malignancy arising from the mesothelial cells lining the body cavities and are rare in domestic animals. In dogs, mesotheliomas have typically been reported in animals of between four and 13 years old (Barker, 1993; Head *et al.*, 2002), although this tumour has been reported in dogs of seven weeks (Leisewitz and Nesbit, 1992) and 11 months (Kim *et al.*, 2002) of age, respectively. The occurrence of this neoplasm in young dogs, as in the current case, suggests these tumours may be congenital in origin as identified in calves (Head *et al.*, 2002). Factors implicated in the pathogenesis of mesothelioma are exposure to asbestos, iron, or silica dust in industrial settings (Barker, 1993; Cicala *et al.*, 1993; Head *et al.*, 2002), in addition to viral or genetic factors (Chabot *et al.*, 1970; Cacciotti *et al.*, 2001; Head *et al.*, 2002). In particular, avian leucosis virus MC 29 infection in chickens (Chabot *et al.*, 1970) and papovavirus SV40 infection in hamsters and rats (Head *et al.*, 2002) have been associated with these tumours. In the current case, on questioning the owner, there was no history of exposure to industrial dusts.

The diagnosis of mesothelioma can prove challenging both in terms of histomorphological appearance and immunohistochemical labelling. The most common type, as in this case, is epitheloid mesothelioma (Barker, 1993; Head *et al.*, 2002) where tumour cells are epitheliod in appearance and form papillary structures. The less frequently reported sarcomatoid type more closely resembles a fibrosarcoma (Head *et al.*, 2002). A biphasic or combination-type neoplasm incorporating features of both types is also described. Mesotheliomas are typically considered a low grade malignancy with minimal tissue invasion and rarely metastasis to drainage lymph nodes or more distant sites (Barker, 1993; Head *et al.*, 2002).

In reaching a diagnosis of epithelioid mesothelioma, metastatic adenocarcinoma must be ruled out. This differentiation is based on eliminating the presence of a primary adenocarcinoma at necropsy and on the use of immunohistochemical labelling. Immunohistochemical markers that can be used to differentiate mesotheliomas from other neoplasms include cytokeratin, vimentin, CEA, calretinin and MFGRA (Epenetos *et al.*, 1982; Whitaker *et al.*, 1982; Battifora and Kopinski, 1985; McCaughey *et al.*, 1985; Nelson and Ordonez, 1989; Ordonez, 2002). Typically, mesotheliomas label with antibodies directed against cytokeratin and vimentin and are negative for CEA (Churg, 1985; Mullink *et al.*, 1986; Azumi and Battifora, 1987; Nelson and Ordonez, 1989; Dias Pereira *et al.*, 2001). Adenocarcinomas are usually cytokeratin positive, vimentin negative and some 70% have been reported as testing positive for CEA (Wang *et al.*, 1979; Corson and Pinkus, 1982; Holden and Churg, 1984; Battifora and Kopinski, 1985; Nelson and Ordonez, 1989). Battifora and Kopinski (1985) reported that labelling for MFGRA is useful in identifying pulmonary and ovarian adenocarcinomas. Antibodies directed against the antigen calretinin have been used in the diagnosis of human mesotheliomas and labelling for this antigen was equivocal in the current case (Nelson and Ordonez, 1989; Geninet *et al.*, 2003). Kutsal *et al.* (2003) reported positive labelling for this antigen in a mesothelioma in a rabbit. It is possible that variations in the results obtained with such immunohistochemical markers in different studies reflects differences in antigen availability within the tumour, which in turn will be influenced by variables such as the degree of tumour differentiation and on the method of tissue fixation (Holthofer *et al.*, 1983; Said, 1983; Said *et al.*, 1983; Churg, 1985; Azumi and Battifora, 1987).

In conclusion, the distribution and macroscopic appearance of the tumour, together with its histopathological features and immunohistochemical labelling characteristics, are consistent with a diagnosis of epitheliod mesothelioma. Such a neoplasm in a young dog is a rare event and its cause remains unclear.

References

Azumi, N. and Battifora, H. (1987). The distribution of vimentin and keratin in epithelial and nonepithelial neoplasms. A comprehensive immunohistochemical study on formalin and alcohol-fixed tumors. *American Journal of Clinical Pathology* **88**: 286-296.

Barker, I.K. (1993). Neoplastic disease of the peritoneum. In: *Pathology of Domestic Animals*. Fourth edition pp 443-445. Edited by K.V.F. Jubb, P.C. Kennedy and N. Palmer. San Diego CA: Academic Press Inc.

Battifora, H. and Kopinski, M.I. (1985). Distinction of mesothelioma from adenocarcinoma. An immunohistochemical approach. *Cancer* **55**: 1679-1685.
Cacciotti, P., Libener, R., Betta, P., Martini, F., Porta, C., Procopio, A., Strizzi, L., Penengo, L., Tognon, M., Mutti, L. and Gaudino, G. (2001). SV40 replication in human mesothelial cells induces HGF/Met receptor activation: a model for viral-related carcinogenesis of human malignant mesothelioma. *Proceedings of the National Academy of Sciences* **98**: 12032-12037.
Chabot, J.F., Beard, D., Langlois, A.J. and Beard, J.W. (1970). Mesotheliomas of peritoneum, epicardium, and pericardium induced by strain MC29 avian leucosis virus. *Cancer Research* **30**: 1287-1308.
Churg, A. (1985). Immunohistochemical staining for vimentin and keratin in malignant mesothelioma. *American Journal of Surgical Pathology* **9**: 360-365.
Cicala, C., Pompetti, F. and Carbone, M. (1993). SV40 induces mesotheliomas in hamsters. *American Journal of Pathology* **142:**1524-1533.
Corson, J.M. and Pinkus, G.S. (1982). Mesothelioma: profile of keratin proteins and carcinoembryonic antigen: an immunoperoxidase study of 20 cases and comparison with pulmonary adenocarcinomas. *American Journal of Pathology* **108**: 80-87.
Cunningham, A.A. and Dhillon, A.P. (1998). Pleural malignant mesothelioma in a captive clouded leopard (*Neofelis nebulosa nebulosa*). *Veterinary Record* **143**: 22-24.
Dias Pereira, P., Azevedo, M. and Gartner, F. (2001). Case of malignant biphasic mesothelioma in a dog. *Veterinary Record* **149**: 680-681.
Epenetos A.A., Canti, G., Taylor-Papadimitriou, J., Curling, M. and Bodmer, W.F. (1982). Use of two epithelium-specific monoclonal antibodies for diagnosis of malignancy in serous effusions. *Lancet* **6**: 1004-1006.
Geninet, C., Bernex, F., Rakotovao, F., Crespeau, F.L., Parodi, A.L. and Fontaine J.J. (2003). Sclerosing peritoneal mesothelioma in a dog - a case report. *Journal of Veterinary Medicine Series A: Physiology, Pathology, Clinical Med*icine **50**: 402-5.
Goelz, M.F., Dixon, D., Clark, J.A. and Myers, P.H. (1993). Diagnostic exercise: pleural and peritoneal nodules in a Fischer 344 rat. *Laboratory Animal Science* **43**: 616-618.
Head, K.W., Else, R.W. and Dubielzig, R.R. (2002). Tumours of the alimentary tract. *In*: D.J. Meuten (ed.) *Tumours In Domestic Animals*. Ames: Iowa State Press. Fourth edition. pp 401-481.
Holden, J. and Churg, A. (1984). Immunohistochemical staining for keratin and carcinoembryonic antigen in the diagnosis of malignant mesothelioma. *American Journal of Surgical Pathology* **8**: 277-279.
Holthofer, H., Miettinen, A., Paasivuo, R., Lehto, V.P., Linder, E., Alfthan, O. and Virtanen I. (1983). Cellular origin and differentiation of renal carcinomas. A fluorescence microscopic study with kidney-specific antibodies, antiintermediate filament antibodies, and lectins. *Laboratory Invest*igation **49**: 317-326.
Kim, J.H., Choi, Y.K., Yoon, H.Y., Kweon, O.K. and Kim, D.Y. (2002). Juvenile malignant mesothelioma in a dog. *Journal of Veterinary Medical Science* **64**: 269-271.
Kutsal, O., Atalay Vural, S., Arslan, H. and Kalınbacak, A. (2003). Concurrent peritoneal mesothelioma and uterine adenocarcinoma in a rabbit. *Journal of the Faculty of Veterinary Medicine University of Kafkasg* **9**: 89-94.
Leisewitz, A.L. and Nesbit, J.W. (1992). Malignant mesothelioma in a seven-week-old puppy. *Journal South African Veterinary Association* **63**: 70-73.
McCaughey, W.T.E., Kannerstein, M. and Churg, J. (1985). *Atlas of Tumor Pathology*. Second edition pp. 1-24 Washington D.C: Armed Forces Institute of Pathology.
Mullink, H., Henzen-Logmans, S.C., Alons-van Kordelaar, J.J., Tadema, T.M. and Meijer, C.J. (1986). Simultaneous immunoenzyme staining of vimentin and cytokeratins with monoclonal antibodies as an aid in the differential diagnosis of malignant mesothelioma from pulmonary adenocarcinoma. *Virchows Archive B: Cell Pathology Including Molecular Pathology* **52**: 55-65.
Nelson, G. and Ordonez, M.D. (1989). The immunohistochemical diagnosis of mesothelioma. *American Journal of Surgical Pathology* **13**: 276-291.
Ordonez, N.G. (2002). Immunohistochemical diagnosis of epithelioid mesotheliomas: a critical review of old markers, new markers. *Human Pathology* **33**: 953-967.
Said, J.W. (1983). Immunohistochemical localization of keratin proteins in tumor diagnosis. *Human Pathology* **14**: 1017-1019.
Said, J.W., Nash, G., Tepper, G. and Banks-Schlegel, S. (1983). Keratin proteins and carcinoembryonic antigen in lung carcinoma: an immunoperoxidase study of fifty-four cases, with ultrastructural correlations. *Human Pathology***14**: 70-76.
Shin, M.L. and Firminger, H.I. (1973). Acute and chronic effects of intraperitoneal injection of two types of asbestos in rats with a study of the histopathogenesis and ultrastructure of resulting mesotheliomas. *American Journal of Pathology* **70**: 291-313.
Wang, N.S., Huang, S.N. and Gold, P. (1979). Absence of carcinoembryonic antigen-like material in mesothelioma: an immunohistochemical differentiation from other lung cancers. *Cancer* **44**: 937-943.
Webster, I., Goldstein, B., Coetzee, F.S. and van Sittert, G.C. (1993). Malignant mesothelioma induced in baboons by inhalation of amosite asbestos. *American Journal of Industrial Medicine* **24**: 659-666.
Whitaker, D., Sterrett, G.F. and Shilkin, K.B. (1982). Detection of tissue CEA-like substance as an aid in the differential diagnosis of malignant mesothelioma. *Pathology* **14**: 255-258.
Yener, Z., Karaca, T. and Yuksel, H. (2002). Abdominal malignant mesothelioma in a mouse. *Australian Veterinary Journal* **80**: 301-302.