Assessment of cytological criteria for diagnosing osteosarcoma in dogs

OBJECTIVES: To evaluate the specific cytological criteria of osteosarcomas in dogs.

METHODS: Significant cytological characteristics of osteosarcoma and benign mesenchymal bone proliferations were determined from imprint smears of 25 dogs with osteosarcoma (group 1) and 20 dogs admitted for removal of surgical bone implants after uncomplicated healing of bone fractures (group 2).

RESULTS: Mild to moderate cellular necrosis occurred frequently in patients with osteosarcoma. The cytoplasm of osteoblasts was pale blue to blue with a more pronounced basophilia in group 2. In 48 per cent of the patients in group 1, but none in group 2, osteoblasts showed a slight to moderate eosinophilic cytoplasmic granulation. In both groups, osteoblasts contained one red to pale blue nucleus with one or two grey-red to blue nucleoli in group 2. Forty-four per cent of animals in group 1 had osteoblasts with more than two nucleoli per nucleus. The median nuclear:cytoplasmic ratio was higher in group 1 (1:2·0) than in group 2 (1:3·5). Osteoblasts in group 1 were frequently seen to have a clumped chromatin pattern and showed significantly more criteria of malignancy (median six criteria per patient) than those in group 2 (median two criteria per patient). In group 1, mitoses of osteoblasts were detectable in 23 of 25 dogs, whereas only one dog in group 2 had evidence of mitotic osteoblasts.

CLINICAL SIGNIFICANCE: Cytological criteria can be helpful in the diagnosis of canine osteosarcoma.

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INTRODUCTION

Osteosarcomas (OSAs) are the most frequently occurring bone tumours in dogs (Brodey and others 1963). Chondrosarcomas, fibrosarcomas, plasma cell tumours and metastatic bone tumours occur less often (Brodey and others 1963, Knecht and Priester 1978, Jongeward 1985). Although OSAs frequently have characteristic clinical and radiological features, diagnosis should always be based on a pathological examination. Several techniques for bone biopsies have been described (Martin and Ellis 1930, Schajowicz and Hokama 1976, Mankin and others 1982) including open surgical biopsy, core biopsy and fine-needle aspiration biopsy (FNAB). Open surgical and core biopsies can be carried out to obtain samples for histological and cytological examination but can only be performed under general anaesthetic, and are associated with more complications (Mankin and others 1982). FNAB can be performed without anaesthesia but the material obtained can only be used for cytological examination (Akerman and others 1976, Mahaffey 1999). In contrast to histology, cytology can be performed without delay (within several minutes) although it may not provide definitive information in all cases.

In humans, FNAB cytology is used with increasing frequency in the diagnosis of bone tumours and is associated with high diagnostic accuracy (Jorda and others 2000). In companion animals, FNAB cytology correlates well with histological examination (Stockhaus and others 2003). However, in humans and companion animals differentiation of benign mesenchymal proliferations and moderate to well-differentiated OSAs is frequently difficult (Jorda and others 2000, Stockhaus and others 2003). Furthermore, subtyping of sarcomas is difficult when based on cytological examination because of the lack of architectural information in cytological specimens (Layfield and others 1987, White and others 1988, Jorda and others 2000, Stockhaus and others 2003).

Cytological criteria in dogs with benign and malignant bone diseases have not been evaluated, making an objective and standardised cytological examination of bone biopsies difficult. The diagnostic criteria which have been described in the veterinary literature are frequently extrapolated from human histology and cytology, and general veterinary cytology (Akerman and others 1976, Mahaffey 1999).

The aim of the study reported here was to determine significant cytological characteristics of OSAs in dogs, which can be used to differentiate benign bone lesions from OSAs.

MATERIALS AND METHODS

Cytological touch impressions of open surgical bone biopsies were performed in 25 dogs with OSA (group 1) and in 20 dogs which were admitted to the Small Animal Clinic at the Free University of Berlin, for removal of bone implants after uncomplicated healing of bone fractures (group 2). Group 1 consisted of 16 different breeds and crossbreeds, aged between one and 13 years (median 7·2 years). Group 2 consisted of 15 breeds and crossbreeds with a median age of two years. Tumours were located in the humerus (n=6), radius (n=6), femur (n=5), skull (n=4), vertebrae (n=2), tibia (n=1) and sternum (n=1). In all cases, tumours were diagnosed by histological examination of open surgical biopsy material. The dogs in group 2 had suffered from bone fractures four weeks to eight months previously (median duration three months). In all cases, there was no evidence of a tumour-associated fracture.

Histological examination

Excised tumour specimens were fixed in 10 per cent neutral buffered formalin. Representative parts of the slices of fixed tissue were then routinely embedded in paraffin wax and 4 µm thick sections were stained with haematoxylin and eosin. Prior decalcification of the tissue slices had been performed as necessary to obtain appropriate sections of hard tumours.

Table 1. Description of general cytological criteria for evaluating bone preparations in dogs

Cytological criteria	Intensity/statistics
Quality	Poor (1)=>40% degenerate cells Moderate (2)=10 to 40% degenerate cells Good (3)=<10% degenerate cells
Cellularity	(0)=hardly any cells Poor (1)=3 to 6 cell aggregates per slide Moderate (2)=7 to 15 cell aggregates per slide High (3)=>15 cell aggregates per slide
Blood contamination	(0)=blood not detectable Low (1)=very small amount Moderate (2)=small amount of blood scattered among cell aggregates High (3)=large amount of blood among cell aggregates
Bacteria	(0)=no bacteria detectable (1)=bacteria present
Necrosis	(0)=no necrosis Mild (1)=<5% cells with necrosis Moderate (2)=5 to 10% cells with necrosis High (3)=>10% cells with necrosis
Osteoid	(0)=no osteoid Mild (1)=small amount of osteoid scattered among osteoblasts High (2)=large amount of osteoid detectable among the osteoblasts
Number of cell populations	%=150 cells of the total cell population counted: osteoblasts fibroblasts, fibrocytes, neutrophils, eosinophils, mast cells, lymphocytes, lymphoblasts, plasma cells, endothelial cells, macrophages, osteoclasts
Haematopoietic precursor cells	(0)=no precursor cells Mild (1)=<5% precursor cells Moderate (2)=5 to 20% precursor cells High (3)=20 to 40% precursor cells

Table 2. Description of nuclear and cytoplasmic criteria of osteoblasts and fibroblasts for evaluating bone preparations

Cytological criteria	Intensity/statistics
Cell borders	(1)=not distinct, (2)=medium, (3)=distinct, mean per 50 cells
Cell size	Mean internal diameter per 50 cells
Cell width	Radial diameter per 50 cells
Nuclear size	Mean length per 50 cells
Nuclear width	Radial diameter per 50 cells
Nucleus:cytoplasm ratio	Mean ratio per 50 cells
Nuclei per cell	Mean number per 100 cells
Nucleoli per nucleus	Mean number per 100 cells
Mitoses	Mean number per 150 cells
Colour of nuclei	Red (1), pale blue (2), dark blue (3), mean per 50 cells
Colour of nucleoli	Grey-red (1), blue (2), dark blue (3), mean per 50 cells
Angular nucleoli	(0)=none Irregular (1)=angular nucleoli in 0 to 5% of the osteoblasts Moderate (2)=detected in 5 to 20% of the osteoblasts High (3)=occur in 20 to 50% of the osteoblasts, mean per 50 cells
Nuclear membrane	(0)=normal Irregular (1)=thickened in <5% of the osteoblasts Mild (2)=thickened membrane in >5% of the cells
Chromatin pattern	(1)=finely stippled, (2)=reticular, (3)=coarse, (4)=clumped, (5)=smudged
Colour of the cytoplasm	(1)=pale blue, (2)=blue, (3)=dark blue, mean per 50 cells
Vacuoles in cytoplasm	(0)=none, (1)=some, (2)=moderate, (3)=many, mean per 50 cells
Malignancy criteria (see Fig 4)	(0)=none, (1)=occurrence per 100 cells, nuclear and cytoplasmic criteria
Eosinophilic granulation of osteoblasts	(0)=none (1)=some cells Intermediate (2)=cells with eosinophilic granulation between osteoblasts without granules Many (3)=>50% granulation
Cell form in fibroblasts	0=none 1=some round fibroblasts (<20%) (2)=many round fibroblasts (>20%)

Cytological examination

In order to obtain the best correlation between cytology and histology, imprint smears of histological biopsies were used for cytological evaluation. Neoplastic bone tissue in group 1 and callus tissue in group

2 were obtained during surgery. Parts of each tissue piece were first pressed on to a piece of paper to reduce the amount of blood on the tissue surface and then multiple impression smears were prepared using glass slides. Other parts of each tumour sample were used for histological examination. The cytological smears were air dried and stained with May-Grünwald-Giemsa stain (Hemafix; Biomed).

During the examinations the smears were coded to prevent the observers from knowing the disease of each dog. The most representative parts of the smears, containing sufficient cellular material, were collected for cytological assessment. The slides were examined for general cytological criteria, including quality, cellularity, presence of bacteria, frequency of cellular degeneration/necrosis, amount of osteoid and incidence of cell populations, including osteoblasts, chondroblasts, chondrocytes, fibroblasts, fibrocytes and inflammatory cells (Table 1). Osteoblasts, osteoclasts and fibroblasts were analysed for general cell criteria, nuclear criteria and cytoplasmic criteria (Table 2).

Statistical analysis

Statistical analysis was performed using the SPSS Windows 10.1 statistical package. For cytological parameters, the mean, 95 per cent confidence interval and relative proportion of the two groups were recorded. The influence of the underlying disease (OSA or reparative callus tissue) on the intensity of cytological parameters was analysed with a one-way analysis of variance for interval data, the Kruskall-Wallis test for ordinal data, and the chi-squared test for bivariate data. $P<0.05$ was considered significant.

RESULTS

Histological examination of bone biopsies in animals with bone tumours revealed three subtypes of OSA, including osteoblastic OSA (n=17), chondroblastic OSA (n=5) and fibroblastic OSA (n=3).

Table 3. Results of general cytological criteria applied to dogs with osteosarcoma (group 1) and dogs with reparative bone tissue (callus) after bone fracture (group 2)

Cytological criteria	Osteosarcoma	Callus	P value
Quality (% of animals)	(1)=20, (2)=80	(1)=30, (2)=65, (3)=5	0·656
Cellularity (% of animals)	(1)=20, (2)=48, (3)=32	(1)=65, (2)=30, (3)=5	0·001
Blood contamination (% of animals)	(1)=24, (2)=68, (3)=8	(1)=10, (2)=60, (3)=30	0·047
Bacteria (% of animals)	(0)=96, (1)=4	(0)=100	0·366
Necrosis (% of animals)	(0)=16, (1)=52, (2)=32	(0)=70, (1)=30,	0·0001
Osteoid (% of animals)	(0)=24, (1)=64, (2)=12	(0)=40, (1)=35, (2)=25	0·792
Haematopoietic precursor cells (% of animals)	(0)=96, (1)=0, (2)=4	(0)=25, (1)=35, (2)=35, (3)=5	0·0001
Osteoblasts (% of total cell population)	Mean=84·7, range=64·97·5	Mean=89·4, range=61·98	0·077
Fibroblasts (% of total cell population)	Mean=9·46, range=0·33	Mean=6·85, range=0·5·36	0·29
Osteoclasts (% of total cell population)	Mean=3·96, range=0·8	Mean=3, range=0·5·7	0·104
Lymphocytes/plasma cells (% of total cell population)	Mean=0·04, range=0·1	Mean=0, range=0·0	0·37
Neutrophils (% of total cell population)	Mean=0·6, range=0·3·5	Mean=0·47, range=0·5	0·32
Macrophages (% of total cell population)	Mean=0·48, range=0·4·5	Mean=0	0·36
Mast cells (% of total cell population)	Mean=0·08, range=0·2	Mean=0	0·37

(0), (1), (2), (3) See descriptions in Tables 1 and 2

The results of general cytological criteria in both groups are shown in Table 3. Quality was moderately good in most cases and was less frequently poor due to cellular degeneration in both groups. Cellularity was higher in the tumour group than in group 2. The specimens in both groups regularly contained moderate amounts of blood. Bacteria were detectable only in one patient with OSA. Cellular necrosis was found in 84 per cent of patients with OSA and in 30 per cent of patients in group 2.

In both groups, mild to moderate amounts of an eosinophilic, osteoid-like, extracellular substance surrounding osteoblasts was detectable in many cases. Haematopoietic precursor cells occurred frequently in specimens from group 2 but were only rarely detectable in group 1. In both groups, osteoblasts (86 to 90·5 per cent) were the most prominent cell type, although several fibroblasts (0 to 36 per cent) could also be found. Less frequently osteoclasts (3 to 4 per cent) and only rarely chondroblasts and chondrocytes (less than 1 per cent each) were present. There was also no significant difference in the relative proportions of other cell populations.

The specific cellular parameters of the different cell populations, including osteoblasts, fibroblasts and osteoclasts, are given in Table 4. Osteoblasts in group 1 dogs frequently had poor to moderately distinct cell borders, while in group 2 cell borders were more distinct. The cytoplasm of osteoblasts was pale blue to blue with a more pronounced basophilia in osteoblasts in dogs from group 2. In 48 per cent of the dogs with OSA, but not in any dogs from group 2, osteoblasts showed a slight to moderate eosinophilic cytoplasmic granulation (Fig 1). In dogs from both groups, osteoblasts contained one red to pale blue nucleus. In group 2 dogs, osteoblasts contained one or two nucleoli while, in group 1, 44 per cent of the dogs had osteoblasts with more than two nucleoli per nucleus. In both groups, nucleoli were in most cases grey-red to blue. The cellular diameter was higher in group 2, while the nuclear diameter was significantly higher in group 1. Therefore, the median nuclear:cytoplasm ratio was higher in group 1 (1:2·0) than in group 2 (1:3·5).

In group 1 dogs, the frequency of mitoses of osteoblasts ranged between 0 and 4 per cent, and only two of 25 dogs with OSAs showed no evidence of mitotic osteoblasts. In contrast, mitosis in osteoblasts in cytological smears was only seen

Table 4. Results of cytological criteria applied to dogs with osteosarcoma (group 1) and dogs with reparative bone tissue after bone fracture (group 2)

Cytological criteria examined in osteoblasts	Osteosarcoma (group 1)		Callus (group 2)		P value
	Mean	95% confidence interval	Mean	95% confidence interval	
Cell borders	1·44	1·23-1·65	2·1	1·96-2·24	0·0001
Cell size (µm)	19·29	18·41-20·17	20·53	19·63-21·4	0·05
Nuclear size	13·01	12·46-13·56	10·7	10·28-11·13	0·0001
Nucleus:cytoplasm ratio	2·07	1·95-2·2	3·56	3·31-3·8	0·0001
Nuclei per cell	1·12	0·98-1·26	1·0	1·0-1·0	0·113
Nucleoli per nucleus	2·44	2·12-2·76	1·4	1·16-1·64	0·000
Mitoses	2·44	1·64-3·24	0·1	0·1-0·1	0·000
Colour of nuclei	1·8	1·51-2·09	1·5	1·26-1·74	0·162
Colour of nucleoli	1·8	1·53-2·07	1·4	1·16-1·64	0·036
Angular nucleoli	0·88	0·58-1·18	0·05	0·05-0·05	0·000
Nuclear membrane	0·44	0·20-0·68	0·05	0·05-0·05	0·007
Chromatin pattern	2·68	2·4-2·96	1·7	1·48-1·92	0·0001
Colour of the cytoplasm	1·56	1·32-1·80	2·05	1·95-2·15	0·001
Vacuoles in cytoplasm	0·44	0·2-0·68	0·05	0·05-0·05	0·007
Malignancy criteria	6·28	5·13-7·43	2·0	1·7-2·3	0·0001
Eosinophilic granulation of osteoblasts	0·56	0·29-0·83	0	0	0·0001
Cell form in fibroblasts	0·75	0·49-1·01	0	0	0·0001

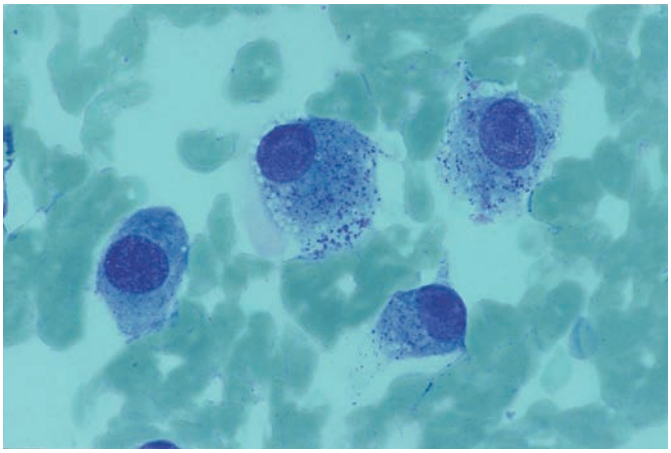

FIG 1. Osteoblasts with cytoplasmic eosinophilic granulation. May-Grünwald-Giemsa. ×1000

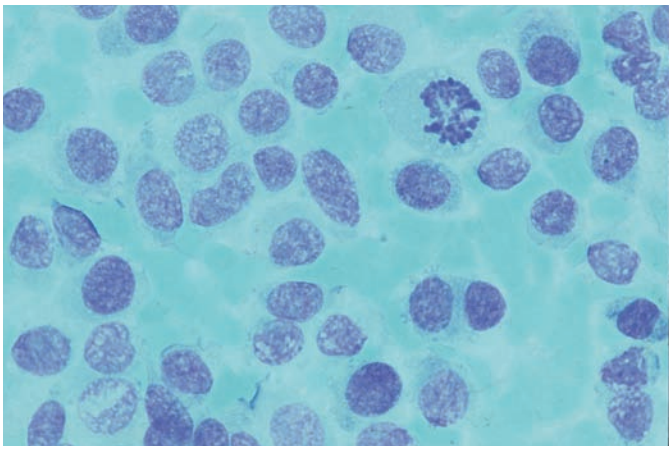

FIG 2. Osteoblasts with aberrant mitosis due to unequal distribution of chromatin. May-Grünwald-Giemsa. ×400

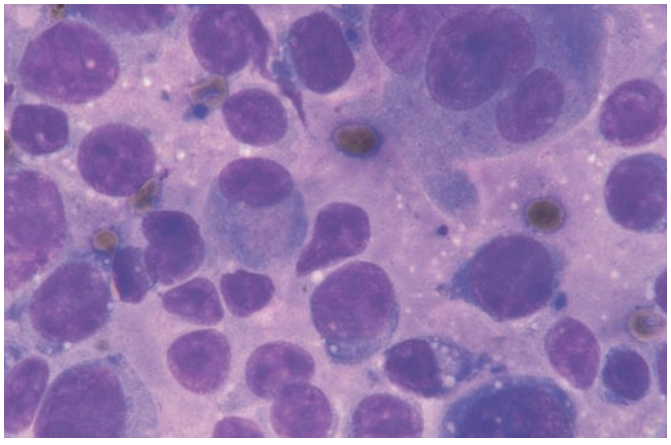

FIG 3. Pleomorphic osteoblasts with clumped chromatin pattern, anisonucleosis and angular nucleoli. May-Grünwald-Giemsa. ×1000

in one dog from group 2. Osteoblasts in group 2 dogs showed a finely stippled to reticular chromatin pattern, while osteoblasts in dogs with OSAs had a reticular to clumped chromatin pattern. Osteoblasts in group 1 dogs had significantly more criteria of malignancy (median six per patient) than osteoblasts in group 2 dogs (median two per patient), including angular nucleoli (68 per cent), anisonucleosis (76 per cent), macronucleolisation (52 per cent), nuclear moulding (48 per cent), aberrant mitoses (44 per cent) including tripolar mitoses or those with unequal distribution of chromatin (Fig 2), thickening of the nuclear membrane (40 per cent), and cytoplasmic vacuolisation (40 per cent) (Figs 3 and 4). Anisocytosis and anisokaryosis were detectable in most cases in both groups. Osteoclasts had similar chromatin patterns (reticular pattern), and frequently showed anisocytosis and anisokaryosis in dogs in both groups.

In contrast to osteoblasts, fibroblasts showed no significant difference in the nuclear:cytoplasm ratio between the two groups. In group 1 dogs, fibroblasts frequently had a round cell form (66 per cent of the dogs) in contrast to fibroblasts in dogs from group 2 which always had an elongated spindle-like cell form. In addition, fibroblasts in dogs from group 1 had more malignancy criteria. However, no criterion was specifically associated with group 1.

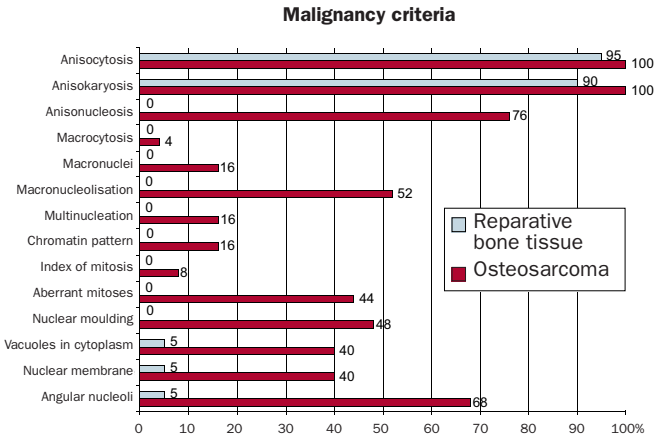

FIG 4. Malignancy criteria of osteoblasts in dogs with osteosarcoma and dogs with reparative bone tissue

DISCUSSION

A preoperative diagnosis is essential in the management of bone tumours including OSA (Den Heeten and others 1985, MacEwen and Kurzman 1996, Jorda and others 2000). In humans, fine-needle aspiration cytology has shown increasing relevance in the diagnosis of bone tumours, having first been described in 1930 by Martin and Ellis. The advantage of this technique is the minimal invasiveness without the need for anaesthesia (El-Khoury and others 1983). In contrast, core-needle biopsies and open surgical biopsies are criticised for their invasiveness, the rate of complications, and the time lapse before a concrete diagnosis can be reached (Mankin and others 1982).

Several studies have pointed out that accuracy in diagnosis of the tumour can reach values above 85 per cent (Akerman and others 1976, Layfield and others 1987, Jorda and others 2000). However, since tissue architecture cannot be interpreted with cytology, subtyping of tumours is frequently not possible. Furthermore, it is difficult to differentiate inflammatory or reactive bone proliferations and OSAs based solely on cytological examination (Stockhaus and Teske 2001, Stockhaus and others 2003).

Several cytological criteria for diagnosing OSA have been described in the veterinary (Mahaffey 1999) and medical literature (Hajdu and Melamed 1971, Akerman and others 1976, Collins and others 1998). However, these criteria have usually been extrapolated from human histology and cytology, and general veterinary cytology. Therefore, the present study was performed to evaluate cytological criteria for non-neoplastic bone lesions and OSA in the dog.

Because of the need for direct comparison of the cytological smears with histology, imprint smears of histological biopsies were used and not FNABs. This might have influenced the parameters of cellularity and the quality of the smears. However, there is no reason to believe that this would have influenced other parameters.

In most dogs with OSA, cellularity was moderate and in reparative bone proliferations cellularity was poor to moderate. This might have been caused by increased cell exfoliation in malignant tumours due to diminished cellular cohesion (Perman and others 1979), while in benign mesenchymal proliferations cellular cohesion is normal (Jayram and others 1994, Stockhaus and others 2003).

In most cases, the quality of the tissue-imprint smears was moderate to good. In FNAB of bone cells, yield can sometimes be poor due to problems in obtaining sufficient cell material (Stockhaus and others 2003).

Haematopoietic precursor cells occurred frequently in callus material but not in malignant tissue. It is probable that physiological haematopoiesis is reduced in tumour areas due to infiltration of tumour tissue.

Smears of OSA tissue frequently contained moderate amounts of necrotic cellular material, probably due to deficient vascularisation of tumour tissue. In OSA tissue and in callus tissue, osteoblasts and fibroblasts were the predominant cell types. Osteoblasts in OSA frequently had poor to moderately distinct cell borders and grey-red to light blue cytoplasm. In this study, cytoplasmic basophilia was more pronounced in callus tissue, although this criterion has been described as a sign of malignancy caused by increased RNA content of neoplastic cells (Zinkl 1981, Tyler and others 1999).

According to the observations of Hajdu and Melamed (1971), eosinophilic granulation of cytoplasm was only detectable in OSA. However, the present study does not prove that granulation reflects a tumour-specific product.

In both groups 1 and 2, osteoblasts contained one pale red to grey-red nucleus. The nuclei contained one or two grey-red to blue nucleoli in callus smears and frequently more than two nucleoli per nucleus in OSA. This observation has been confirmed in humans with OSA (Jayram and others 1994, Collins and others 1998). In OSA tissue, the nucleus:cytoplasm ratio was significantly increased, as a result of an increase in the nuclear diameter and a decrease in the cellular diameter, compared with that in dogs with reparative proliferations. In malignant tumours, the nuclear:cytoplasm ratio is frequently lower than 1:2 because of an increase in nuclear material (Tyler and others 1999). The reduced cellular size of osteoblasts might be related to cellular crowding of tumour cells.

Another important diagnostic criterion in the diagnosis of OSA is the nuclear chromatin pattern which is frequently more clumped and condensed than in benign proliferations (Collins and others 1998).

Osteoblasts in dogs in group 1 showed significantly more criteria of malignancy than osteoblasts in dogs in group 2, including angular nucleoli, thickening of the nuclear membrane, cytoplasmic vacuolisation, anisonucleosis, macronucleolisation, aberrant mitoses and nuclear moulding. The malignancy criteria of anisocytosis, anisokaryosis and cytoplasmic basophilia (Tyler and others 1999) appear not to be relevant parameters in OSA since they occurred frequently in both groups. Osteoclasts were detectable in both groups with similar frequencies and therefore are not specific for the diag-

nosis of OSA. However, in this study, specific methods for the differentiation of benign and malignant giant cells, as described by Jösten and Rudolph (1997) for paraffin wax sections, were not used.

Fibroblasts in OSA tissue showed more criteria of malignancy, including anisocytosis and anisokaryosis, and frequently had a round cell form that has been described in fibrosarcoma in dogs and cats (Stirtzinger 1988). However, it remains uncertain whether this criterion is confined to specific forms of OSA or can be generally observed in all forms of OSA, as the numbers of the subtypes (other than osteoblastic OSA) were too small to expect significant differences. Furthermore, it cannot be completely ruled out that some cells in this study that were identified as fibroblasts were not in fact chondroblasts. However, chondroblasts are frequently more intensely basophilic with the haematological stains used in this study, and the cytoplasm frequently contains cytoplasmic vacuoles (Peng and Yan 1985, Stirtzinger 1988).

Although the cytological determination of malignancy in OSA might be associated with high diagnostic accuracy, it is frequently difficult to identify the exact type of sarcoma based on cytology alone (White and others 1988, Stockhaus and others 2003). Several authors recommend the identification of osteoid as a good criterion for the diagnosis of OSA (Mahaffey 1999). In the present study, extracellular eosinophilic material surrounding osteoblasts, identified as osteoid, was not detectable in 24 per cent of the OSA and 40 per cent of the reparative lesions. Therefore, the cytological identification of a malignant mesenchymal tumour as OSA, based on the occurrence of osteoid (Hajdu and Melamed 1971, Ayala and others 1995), is not a specific procedure. Furthermore, it might be difficult to differentiate collagen, chondroid and osteoid based solely on a haematological stain like May-Grünwald-Giemsa (White and others 1988).

In the present study, most cases of OSA were of intermediate or poor differentiation. Therefore, further studies are needed to determine the value of these cytological criteria and the diagnostic accuracy of the cytology in well-differentiated OSA, which is difficult to diagnose using cytology alone (Kabukcuoglu and others 1998, Jorda and others 2000). Furthermore, it is unclear whether the subtype of OSA may influence the cytological characteristics of the different cell types.

Conclusions

The cytological criteria presented in this study can be helpful in the objective diagnosis of canine OSA but further studies are needed to determine the sensitivity and specificity of cytology in the diagnosis of OSA.

References

Akerman, M., Berg, N. O. & Persson, B. M. (1976) Fine needle aspiration biopsy in the evaluation of tumor-like lesions of bone. *Acta Orthopaedica Scandinavica* **47**, 129-136

Ayala, A. G., Ro, J. Y., Fanning, C. V., Flore, J. P. & Yasko, A. W. (1995) Core needle biopsy and fine needle aspiration in the diagnosis of bone and soft tissue lesions. *Hematology/Oncology Clinics of North America* **9**, 633-651

Brodey, R. S., Sauer, R. M. & Medway, W. (1963) Canine bone neoplasms. *Journal of the American Veterinary Medical Association* **186**, 56-61

Collins, B. T., Cramer, H. M. & Ramos, R. R. (1998) Fine needle aspiration biopsy of recurrent and metastatic osteosarcoma. *Acta Cytologica* **42**, 357-361

Den Heeten, G. J., Oldoff, J., Oosterhuis, H. & Schraffordt Koops, H. (1985) Biopsy of bone tumors. *Journal of Surgical Oncology* **28**, 247-251

El-Khoury, G. Y., Terepka, R. H., Mickelson, M. R., Rainville, K. L. & Zaleski, M. S. (1983) Fine-needle aspiration biopsy of bone. *Journal of Bone and Joint Surgery* **65**, 522-525

Hajdu, S. & Melamed, M. (1971) Needle biopsy of malignant bone tumors. *Surgery, Gynecology & Obstetrics* **133**, 829-832

Jayram, G., Miac, M. D. & Gupta, M. (1994) Fine needle aspiration cytology in the diagnosis of bone tumors. *Malaysian Journal of Pathology* **16**, 137-144

Jongeward, S. J. (1985) Primary bone tumors. *Veterinary Clinics of North America: Small Animal Practice* **15**, 609-641

Jorda, M., Rey, L., Hanly, A. & Ganjei- Azar, P. (2000) Fine needle aspiration cytology of bone. Accuracy and pitfalls of cytodiagnosis. *Cancer Cytopathology* **90**, 47-54

Jösten, M. & Rudolph, R. (1997) Methods for the differentiation of giant cells in canine and feline neoplasias in paraffin sections. *Journal of Veterinary Medicine A* **44**, 159-166

Kabukcuoglu, F., Kabukcuoglu, Y., Kuzgun, Y. & Evren, I. (1998) Fine needle aspiration of malignant bone lesions. *Acta Cytologica* **42**, 875-882

Knecht, C. D. & Priester, W. A. (1978) Musculoskeletal tumors in dogs. *Journal of the American Veterinary Medical Association* **172**, 72-74

Layfield, L. J., Glasgow, B. J., Anders, K. H. & Mirra, J. M. (1987) Fine needle aspiration cytology of primary bone lesions. *Acta Cytologica* **31**, 177-184

MacEwen, E. G. & Kurzman, I. D. (1996) Canine osteosarcoma: amputation and chemoimmunotherapy. *Veterinary Clinics of North America: Small Animal Practice* **26**, 123-133

Mahaffey, E. A. (1999) Cytology of the musculoskeletal system. In: Diagnostic Cytology and Haematology in the Dog and Cat. Eds R. L. Cowell, R. D. Tyler and J. H. Meinkoth. Mosby, St Louis. pp 120-124

Mankin, H. J., Lange, T. A. & Spanier, S. S. (1982) The hazards of biopsy in patients with malignant primary bone and soft-tissue tumors. *Journal of Bone and Joint Surgery* **64**, 1121-1127

Martin, H. E. & Ellis, E. B. (1930) Biopsy by needle puncture and aspiration. *Annual Surgery* **92**, 169-181

Peng, X. & Yan, X. (1985) Cytodiagnosis of bone tumors by fine needle aspiration. *Acta Cytologica* **29**, 570-575

Perman, V., Alsaker, R. D. & Riis, R. C. (1979) Cytology of the Dog and Cat. American Animal Hospital Association, South Bend, Indiana. pp 1-4

Schajowicz, F. & Hokama, J. (1976) Aspiration (puncture or needle) biopsy in bone lesions. *Recent Results in Cancer Research* **54**, 139-144

Stirtzinger, T. (1988) The cytologic diagnosis of mesenchymal tumors. *Seminars in Veterinary Medicine and Surgery (Small Animals)* **3**, 157-166

Stockhaus, C., Schoon, H. S., Scharvogel, S. & Teske, E. (2003) Die Bedeutung der Zytologie für den Nachweis von Knochentumoren bei Hund und Katze. *Tierärztliche Praxis* **31** (K), 117-123

Stockhaus, C. & Teske, E. (2001) Klinische Erfahrungen mit der zytologischen Diagnostik beim Hund. *Schweizer Archiv für Tierheilkunde* **143,** 233-240

Tyler, R. D., Cowell, R. L., Baldwin, C. J. & Morton, R. J. (1999) Introduction. In: Diagnostic Cytology and Haematology in the Dog and Cat. Eds R. L. Cowell, R. D. Tyler and J. H. Meinkoth. Mosby, St Louis. pp 1-19

White, V., Fanning, C. V., Ayala, A. G., Raymond, A. K., Carrasco, C. H. & Murray, J. A. (1988) Osteosarcoma and the role of fine needle aspiration: a study of 51 cases. *Cancer* **62**, 1238-1246

Zinkl, L. (1981) Criteria of malignancy in cytologic preparations. *California Veterinary Journal* **6**, 13-15

Inheritance of dermoid sinus in the Rhodesian ridgeback

OBJECTIVES: To define the mode of inheritance of dermoid sinus.

METHODS: A chi-squared analysis was performed on data from 46 litters produced between 1990 and 2001. Data were corrected to avoid bias in the segregation ratio.

RESULTS: In data from 57 litters (n=492), 82 dermoid sinus positive offspring were observed. The frequency of affected offspring in the Swedish Rhodesian ridgeback population is estimated to be between 8 and 10 per cent.

CLINICAL SIGNIFICANCE: Bias in heredity pattern may be caused by undetected dermoid sinus type V. Improved clinical diagnosis of all dermoid sinus types is therefore crucial.

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INTRODUCTION

The Rhodesian ridgeback is an African dog breed, the characteristic phenotype of which is a coat that forms a dorsal ridge. The ancestry of this phenotype is suggested to be an indigenous African breed – the Hottentot hunting dog (Hare 1932, Hawley 1984). Crossbreed dogs, which originated from European breeds, were mated with the Hottentot hunting dog over a 200-year period, resulting in today's Rhodesian ridgeback breed (Mann and Stratton 1966). The Rhodesian ridgeback breed is often associated with hereditary abnormality referred to as dermoid sinus (Hathcock and others 1979, Lambrechts 1996, Cornegliani and others 2001).

Dermoid sinus is a congenital malformation caused by an incomplete separation of the skin and neural tube during embryonic development (Fatone and others 1995, Booth 1998, Cornegliani and others 2001). The abnormality is also termed a dermoid cyst or pilonidal cyst (Tshamala and Moens 2000). However, there are anatomical differences between a dermoid sinus and a dermoid cyst (Blood and Studdert 1999). A dermoid sinus is a tube-like tract lined by hair follicles and sweat and sebaceous glands (Swenson 1989, Tshamala and Moens 2000). The lumen of the sinus may also contain sebum, hair (Lambrechts 1996, Cornegliani and others 2001) and keratin debris (Scott and others 1995). The composition of the sinus is unchanged through the epidermis, dermis and lower layers of tissue. In contrast, a dermoid cyst is a closed epithelium-lined sac or capsule containing a semi-solid or liquefied substance (Goldsmith and Shofer 1992).

A dermoid sinus connected to the skin can be detected by palpation. If the sinus tube develops a secondary infection, the dog may show signs of pain during palpation (Fatone and others 1995, Lambrechts 1996). One of the characteristics of a dermoid sinus is tufts of hair protruding from single or multiple small skin openings (Hathcock and others 1979). Common anatomical locations of dermoid sinuses are the dorsal cervical, thoracic and coccygeal areas, in other words, before and after the dorsal ridge.

Dermoid sinus occurrence has been reported in American cocker spaniels (Bailey and others 2001), boerboels (Penrith and van Shouwenburg 1994), boxers (Selcer and others 1984), chow-chows (Booth 1998), English cocker spaniels (Pratt and others 2000), golden retrievers (Cornegliani and others 2001), Rhodesian ridgebacks (Hofmeyr 1963, Hathcock and others 1979, Gammie 1986), Rhodesian ridgeback crosses (Lambrechts 1996), shih tzus (Selcer and others 1984), Siberian huskies (Cornegliani and Ghibaudo 1999) and Yorkshire terriers (Fatone and others 1995).

Different types of dermoid sinus have been described that are classified according to how deeply they penetrate towards the spine (Gammie 1986), in other words their relationship to the supraspinous ligament (Tshamala and Moens 2000), and whether or not an opening in the skin exists. Four types of sinus have been described (Mann and Stratton 1966). Booth (1998) suggested a fifth type of dermoid sinus in Rhodesian ridgebacks, which was subsequently supported by Tshamala and Moens (2000) (Fig 1). The five types of dermoid sinus and the diagnostic features of each are

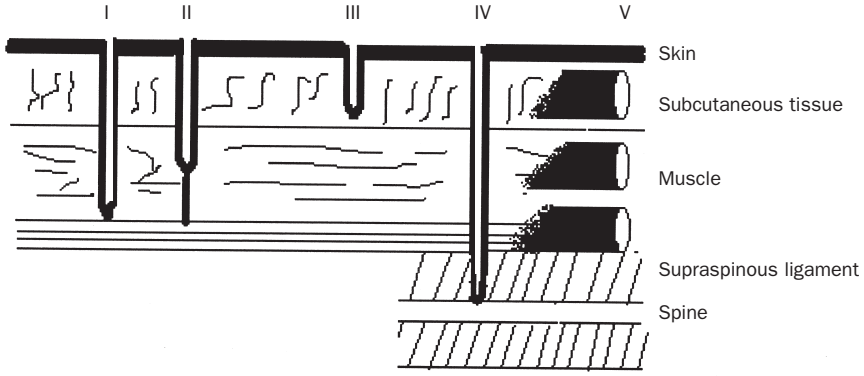

FIG 1. Five types of dermoid sinus. The classification depends on how deeply into tissue the sinus extends and whether or not a skin opening exists. Dermoid sinus type V lacks a skin opening or a definite location in a specific tissue. The diagram shows a midline longitudinal section demonstrating the different types of dermoid sinus. The original image was adapted from Mann and Stratton (1966) and has been edited by the author

Table 1. Diagnostic features of the five types of dermoid sinus

Dermoid sinus type	Features
I	Extending as far as the supraspinous ligament, and presumably nuchal ligament in the cervical area, to which it is attached. The nuchal ligament is an elastic apparatus that serves to support the head without muscular effort (Blood and Studdert 1999)
II	Not extending as far as the supraspinous ligament but connected to it by a fibrous strand
III	Not extending as far as, or connected to, the supraspinous ligament
IV	Attached to the dura mater
V	Dermoid sinus with no connection to the skin surface (Booth 1998, Tshamala and Moens 2000). No definite location in a specific tissue

listed in Table 1. The fifth type has no connection to the skin and is therefore difficult to detect via palpation of the skin. When sinus type V is located close to the spine, neurological deficiencies in the hindlimbs, behavioural changes and incontinence have been described (Booth 1998).

Mode of inheritance

Dermoid sinus in Rhodesian ridgebacks has been suggested to be inherited either as a simple autosomal recessive trait (Lord and others 1957, Mann and Stratton 1966, Angarano and Swaim 1993, Scott and others 1995), a dominant trait with incomplete penetration (Hofmeyr 1963, Unwin 1987) or a combination of two recessive genes (Mann and Stratton 1966). The first statistical trial to determine the inheritance of dermoid sinus was performed by Hare in 1932. In that study, all data were retrieved from only two sires and three dams. They produced 47 offspring. One of the sires was dermoid sinus positive. The dermoid sinus negative sire gave rise to a total of 41 offspring, and five were identified as dermoid sinus positive. The dermoid sinus affected sire gave rise to a total of six offspring, and two were dermoid sinus positive. Hare presented no opinion on the statistical results.

In 1957, Lord and others reported the mode of inheritance as being of a complex nature and not simple recessive, based upon the frequency with which dermoid sinus appeared. Hofmeyr (1963) suggested that dermoid sinus was of a dominant nature with incomplete penetration, referring to the report written by Lord and others (1957). No statistical evidence was supplied by either Lord and others (1957) or Hofmeyr (1963).

The data by Hare (1932) were re-evaluated by Mann and Stratton in 1966. They concluded that the mode of inheritance was of a simple recessive character. Stratton collected data from breeders relating to 17 sires and 21 dams. One dam was dermoid sinus positive. The matings resulted in 48 litters with a total of 376 offspring. When an analysis was performed using only the litters in which dermoid sinus had been detected (20 litters), the expected number of dermoid sinus positive offspring was 45. The actual outcome was 42 dermoid sinus positive offspring. This analysis supports the hypothesis of a simple autosomal recessive trait (Swenson 1989).

In order to better define the mode of inheritance of dermoid sinus, the work reported in the present study more extensively evaluates the incidence of dermoid sinus in the Swedish Rhodesian ridgeback population.

MATERIALS AND METHODS

Swedish Rhodesian ridgeback breeders have been reporting the occurrence of dermoid sinus to the Swedish Rhodesian Ridgeback Club since 1964. The register from the club is official and available to the public. The Swedish Rhodesian ridgeback population comprises approximately 2500 animals (Swedish Kennel Club 2000 to 2002). The breeding population during the period 1995 to 2001 was 305 animals (171 dams and 134 sires).

Data

Data were collected from the Swedish Rhodesian Ridgeback Club and refer to a total of 129 litters (1040 offspring) derived from matings between 103 sires and 106 dams over an 11-year period (1990 to 2001). The litters included in the study were those in which culling of offspring was exclusively due to dermoid sinus. The author performed the categorisation of the material. None of the parent animals included in this study were recorded as dermoid sinus positive.

The data were analysed from two different angles. One analysis was performed based on the litters where dermoid sinus positive offspring (nn) had been produced and the other analysis was performed based on the classified heterozygote parent animals. Correction of litter data was performed to avoid bias in the segregation ratio, as some parent carriers produced litters where all offspring were dermoid sinus negative (NN or Nn). The correction formula used was $q' = q/(1-p^s)$, where q is the expected frequency of nn (0·25), p is the expected frequency of NN or Nn (1-0, 25), q′ is the corrected expected frequency of nn and s is the litter size (Cavalli-Sforza and Bodmer 1971). When applying the correction formula to the litter data, it was presumed that the parent animals were phenotypically normal heterozygote carriers. Standard chi-squared analysis was performed to test the null hypothesis that data did not deviate from the expected 3:1 distribution of unaffected and affected animals.

RESULTS

Recordings of 72 litters showed no evidence of dermoid sinus positive offspring (a total of 548 individuals). Nine of 62 dams and 22 of 58 sires had produced dermoid

Table 2. Corrected data for 46 litters (from a total of 57 litters produced by classified heterozygote parent animals) in which dermoid sinus positive offspring were identified. Correction of the data was performed to avoid bias in the segregation ratio. The data covers litters produced during 1990 to 2001

Litter number	Sire	Dam	Number observed		Expected			
					Uncorrected		Corrected	
			Normal	Defect	Normal	Defect	Normal	Defect
73	Y59	X63	7	1	6	2	5·78	2·22
74	Y60	X64	8	2	7·5	2·5	7·35	2·65
75	Y60	X64	10	0	–	–	–	–
76	Y61	X65	10	0	–	–	–	–
77	Y62	X66	4	4	6	2	5·78	2·22
78	Y63	X67	7	5	9	3	8·90	3·10
79	Y63	X67	8	0	–	–	–	–
80	Y64	X68	7	1	6	2	5·78	2·22
81	Y65	X69	7	3	7·5	2·5	7·35	2·65
82	Y66	X70	9	2	8·25	2·75	8·13	2·87
83	Y67	X71	9	1	7·5	2·5	7·35	2·65
84	Y68	X9	2	0	–	–	–	–
85	Y68	X9	4	2	4·5	1·5	4·18	1·82
86	Y69	X71	9	0	–	–	–	–
87	Y70	X72	10	1	8·25	2·75	8·13	2·87
88	Y71	X73	10	1	8·25	2·75	8·13	2·87
89	Y72	X74	5	1	4·5	1·5	4·18	1·82
90	Y72	X75	2	2	3	1	2·54	1·46
91	Y73	X70	9	0	–	–	–	–
92	Y74	X73	7	1	6	2	5·78	2·22
93	Y75	X74	10	1	8·25	2·75	8·13	2·87
94	Y76	X75	11	1	9	3	8·90	3·10
95	Y77	X76	9	1	7·5	2·5	7·35	2·65
96	Y77	X76	11	2	9·75	3·25	9·67	3·33
97	Y78	X77	7	1	6	2	5·78	2·22
98	Y79	X20	2	2	3	1	2·54	1·46
99	Y80	X49	10	0	–	–	–	–
100	Y80	X78	10	0	–	–	–	–
101	Y81	X79	3	1	3	1	2·54	1·46
102	Y82	X80	6	5	8·25	2·75	8·13	2·87
103	Y83	X81	6	1	5·25	1·75	4·98	2·02
104	Y84	X78	10	2	9	3	8·90	3·10
105	Y85	X51	6	2	6	2	5·78	2·22
106	Y86	X79	7	1	6	2	5·78	2·22
107	Y87	X80	7	2	6·75	2·25	6·57	2·43
108	Y88	X81	6	1	5·25	1·75	4·98	2·02
109	Y89	X65	5	2	5·25	1·75	4·98	2·02
110	Y89	X82	3	5	6	2	5·78	2·22
111	Y90	X83	7	1	6	2	5·78	2·22
112	Y90	X84	12	1	9·75	3·25	9·67	3·33
113	Y90	X85	9	1	7·5	2·5	7·35	2·65
114	Y90	X86	6	2	6	2	5·78	2·22
115	Y91	X80	9	2	8·25	2·75	8·13	2·87
116	Y92	X73	11	0	–	–	–	–
117	Y93	X87	6	2	6	2	5·78	2·22
118	Y94	X77	5	0	–	–	–	–
119	Y95	X88	8	0	–	–	–	–
120	Y95	X58	2	1	2·25	0·75	1·70	1·30
121	Y96	X89	8	2	7·5	2·5	7·35	2·65
122	Y97	X49	8	2	7·5	2·5	7·35	2·65
123	Y98	X90	8	1	6·75	2·25	6·57	2·43
124	Y99	X91	1	1	1·5	0·5	0·86	1·14
125	Y100	X92	6	3	6·75	2·25	6·57	2·43
126	Y101	X93	6	1	5·25	1·75	4·98	2·02
127	Y102	X75	9	2	8·25	2·75	8·13	2·87
128	Y103	X94	8	2	7·5	2·5	7·35	2·65
129	Y103	X95	8	1	6·75	2·25	6·57	2·43
Sum	**45**	**44**	**410**	**82**	**300**	**100**	**290·0**	**110·0**

Normal An individual that has not been reported as dermoid sinus positive
Defect An individual that has been reported as dermoid sinus positive

sinus positive offspring in previous mating combinations. The nine dams and 22 sires were classified as heterozygote Nn. The remaining parent animals had no identified dermoid sinus positive offspring in previous combinations and were classified as homozygote NN or heterozygote Nn.

In recordings from 57 litters, 46 litters showed dermoid sinus positive offspring. All parent animals (45 sires and 44 dams) were classified as heterozygote Nn. The observed numbers of dermoid sinus positive offspring were 82, whereas 410 individuals were defined as phenotypically normal.

Chi-squared tests were performed on data from the 46 litters (Table 2) in which dermoid sinus positive offspring had been identified. This showed that χ^2 (uncorrect. expected)=43;57, P<0·05, and χ^2 (correct. expected)=56;75, P<0·05.

Based on reported and unreported cases, the frequency of dermoid sinus in this Rhodesian ridgeback population was assumed to range between 8 and 10 per cent.

DISCUSSION

The Swedish Rhodesian ridgeback register may be unique. The objective of the Swedish Rhodesian Ridgeback Club is to register all animals, as well as the health status of produced litters. The vast amount of information in the register allows not only classifications of parent animals (according to the hypothesis), but also enables analysis of the outcome of produced litters. It must be emphasised that the data concerning dermoid sinus appearance were reported by breeders to the Swedish Rhodesian Ridgeback Club and therefore the results entirely rely upon the accuracy of the breeders' information. Some individuals that are born with incorrect ridges, or ridgeless, are culled and normally not examined for dermoid sinus. Stillborn individuals are normally not examined either. As a consequence, a vital piece of information concerning dermoid sinus frequency is lost.

There is no information in earlier reports (Hare 1932, Mann and Stratton 1966) on whether individuals culled for reasons other than dermoid sinus have been excluded from statistical trials. To avoid bias caused by these problems, the litters included in the present study were those where dermoid sinus was the single reported reason for culling.

It is presumed that the information of litter status given by breeders to the Swedish Rhodesian Ridgeback Club was noted consecutively. From the total amount of litters included, 83 (64 per cent) of the litters showed no identified dermoid sinus positive offspring, according to breeders. The observed number of

dermoid sinus positive offspring produced during 1990 to 2001 was 82. Subsequently, an analysis was performed only on those litters where dermoid sinus positive offspring had been detected (82 individuals).

Results from the chi-squared tests performed on the litter data did not support the hypothesis of a simple autosomal recessive mode of inheritance. In other words, there were significant deviations between observed and expected numbers of dermoid sinus positive offspring. Available data instead suggested a more complex dihybrid mode of inheritance. Unfortunately, the currently available material cannot formally prove such a mode of inheritance. There are two possible explanations for the deviations between the observed and corrected expected numbers of dermoid sinus positive offspring (Table 2). Some individuals are not identified as dermoid sinus positive until they have become older and secondary infection has occurred. These individuals are normally not included in the Swedish Rhodesian ridgeback register, since there are no frequent updates concerning the health status of produced litters. Thus, animals that are diagnosed as dermoid sinus positive later in life will influence dermoid sinus frequency. This is an explanation supported by the breeding committee of the Swedish Rhodesian Ridgeback Club.

A rather controversial explanation could be that the existence of sinus type V has remained undetected. Homozygote recessive individuals may not be identified as dermoid sinus affected. As a consequence, individuals affected by type V dermoid sinus could incorrectly be included in the data where parent animals are presumed to be homozygote NN or heterozygote Nn (litters with no apparent dermoid sinus positive individuals) instead of the data in Table 2 (litters with observed dermoid sinus positive individuals). Based on the current study, and considering the probable explanations for the deviations between observed and expected numbers of dermoid sinus positive offspring, the hypothesis of a recessive mode of inheritance cannot be formally excluded. In addition, differential penetrance of the disease appears to be consistent with the clinical phenotype of multiple types of dermoid sinus observed (Fig 1). Thus, a reasonable hypothesis is that a major disease gene, the activity of which is influenced by modifying genes, may play a role during the developmental stages of dermoid sinus. It is important that Rhodesian Ridgeback breeders are informed of the existence of the fifth sinus type (with no skin opening) in an effort to develop diagnostic procedures to detect this type of dermoid sinus.

The definition of a dermoid sinus is based on histological findings throughout this paper, and type V dermoid sinus may have escaped detection (as discussed above). If some of the animals classified as dermoid sinus negative were used for breeding, but were actually positive for the undetected dermoid sinus type V, attempts to identify mode of inheritance via pedigrees would not be completely reliable.

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References

Angarano, D. W. & Swaim, S. F. (1993) Congenital skin diseases. In: Disease Mechanisms in Small Animal Surgery. 2nd edn. Ed M. J. Bojrab. Lea & Febiger, Philadelphia. pp 178-183

Bailey, T. R., Holmaberg, D. L. & Yager, J. A. (2001) Nasal dermoid sinus in an American cocker spaniel. *Canadian Veterinary Journal* **42**, 213-215

Blood, D. C. & Studdert, V. P. (1999) Saunders Comprehensive Veterinary Dictionary. 2nd edn. W. B. Saunders, London. pp 305, 329

Booth, M. J. (1998) Atypical dermoid sinus in a chow chow dog. *Journal of the South African Veterinary Association* **69**, 102-104

Cavalli-Sforza, L. L. & Bodmer, W. F. (1971) The Genetics of Human Populations. W. H. Freeman, San Francisco

Cornegliani, L. & Ghibaudo, G. (1999) A dermoid sinus in a Siberian husky. *Veterinary Dermatology* **10**, 47-49

Cornegliani, L., Jommi, E. & Vercelli, A. (2001) Dermoid sinus in a golden retriever. *Journal of Small Animal Practice* **42**, 514-516

Fatone, G., Brunetti, A., Lamagna, F. & Potena, A. (1995) Dermoid sinus and spinal malformations in a Yorkshire terrier: diagnosis and follow-up. *Journal of Small Animal Practice* **36**, 178-180

Gammie, J. S. (1986) Dermoid sinus removal in a Rhodesian ridgeback dog. *Journal of the South African Veterinary Association* **27**, 250-251

Goldsmith, M. H. & Shofer, F. S. (1992) Cysts. In: Skin Tumors of the Dog and Cat. Pergamon, Oxford. pp 271-283

Hare, T. (1932) A congenital abnormality of hair follicles in dogs resembling trichostasis spinulosa. *Journal of Pathology and Bacteriology* **35**, 569-571

Hathcock, T. J., Clampett, G. E. & Broadstone, V. R. (1979) Dermoid sinus in a Rhodesian ridgeback. *Veterinary Medicine, Small Animal Clinician* **74**, 53-56

Hawley, T. C. (1984) The Hottentot dog. In: The Rhodesian Ridgeback. The Origin, History and Standard. 4th edn. N. G. Sendingpers, Bloemfontein. pp 11-12

Hofmeyr, C. F. B. (1963) Dermoid sinus in the ridgeback dog. *Journal of Small Animal Practice* **4**, 5-8

Lambrechts, N. (1996) Dermoid sinus in a crossbred Rhodesian ridgeback dog involving the second cervical vertebra. *Journal of the South African Veterinary Association* **67**, 155-157

Lord, L. H., Cawley, A. J. & Gilray, J. (1957) Mid-dorsal dermoid sinuses in Rhodesian ridgeback dogs – a case report. *Journal of the American Veterinary Medical Association* **131**, 515-518

Mann, G. E. & Stratton, J. (1966) Dermoid sinus in the Rhodesian ridgeback. *Journal of Small Animal Practice* **7**, 631-642

Penrith, M-L. & Van Shouwenburg, S. (1994) Dermoid sinus in a Boerboel bitch. *Journal of the South African Veterinary Association* **65**, 38-39

Pratt, J. N. J., Knottenbelt, C. M. & Welsh, E. M. (2000) Dermoid sinus at the lumbosacral junction in an English springer spaniel. *Journal of Small Animal Practice* **41**, 24-26

Scott, D. W., Miller, W. H. & Griffin, G. E. (1995) Congenital and hereditary defects. In: Small Animal Dermatology. 5th edn. Eds D. W. Scott, W. H. Miller and G. E. Griffin. W. B. Saunders, Philadelphia. pp 736-805

Selcer, E. A., Helman, R. G. & Selcer, R. R. (1984) Dermoid sinus in a shih tzu and a boxer. *Journal of the American Animal Hospital Association* **20**, 634-636

Swedish Kennel Club (2000-2002) Registration Statistics – Rhodesian ridgeback. *Journal of the Swedish Kennel Club*, **1-2**

Swenson, L. (1989) Ärftlig dermoid sinus hos Rhodesian ridgeback. *Doggy Rapport* **1**, 5

Tshamala, M. & Moens, Y. (2000) True dermoid cyst in a Rhodesian ridgeback. *Journal of Small Animal Practice* **41**, 352-353

Unwin, D. D. (1987) The dermoid sinus. In: Rhodesian Ridgeback. Ed A. Woodrow. Information Printing, Oxford. pp 155-159

Cerebral and conjunctival haemorrhages associated with von Willebrand factor deficiency and canine angiostrongylosis

A case of angiostrongylosis is described in a 14-month-old golden retriever bitch. Conjunctival haemorrhage and neurological signs, referable to a space-occupying cerebral lesion, were associated with defective primary haemostasis caused by low levels of von Willebrand factor. Full clinical recovery followed treatment with desmopressin, fresh whole blood transfusion, fenbendazole and supportive care. The magnetic resonance image of the suspected organising haematoma is described. Similarities to the human condition, acquired von Willebrand syndrome, and a possible role for aberrant larval migration in haematoma formation are suggested.

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INTRODUCTION

Infection with *Angiostrongylus vasorum* is of increasing importance to small animal practitioners. The distribution of cases in the UK may extend as far north as southern Scotland (N. Carmichael, personal observations). Consumptive and immune-mediated haemorrhagic diatheses have been reported (Schelling and others 1986, Ramsey and others 1996, Gould and McInnes 1999, Cury and others 2002b). Initial work characterising the immune response to this parasite in experimental infection has also been published (Cury and others 2002a). This report describes diagnosis and successful management of a previously unreported coagulopathy associated with canine angiostrongylosis.

CASE HISTORY

A 14-month-old, female golden retriever was referred with a four-day history of conjunctival haemorrhage, suspected seizure activity and paroxysmal episodes of poorly localised pain manifested by vocalisation and rigidity of the limbs. The animal's physical status deteriorated rapidly on the day of referral. On physical examination, the dog was non-ambulatory and very weak. A menace response was absent on the right side. Slow, horizontal nystagmus was present with the fast phase towards the left. The white of the sclera was obliterated in both eyes by conjunctival haemorrhage (Fig 1a). Respiratory rate was 80 breaths/minute. Based on a normal platelet count, one-stage prothrombin time (OSPT) and activated partial thromboplastin time (APTT) taken on the day prior to referral, a defect in primary haemostasis was suspected. A buccal mucosal bleeding time (BMBT) was obtained and bleeding persisted at 30 minutes after the initial incision. Ten minutes after administration of desmopressin (DDAVP, 4 µg/kg diluted in 20 ml saline and administered intravenously over 10 minutes), BMBT was three minutes, suggesting von Willebrand factor (VWF) deficiency as the cause of the coagulopathy. VWF levels prior to desmopressin administration were subsequently shown to be 22 per cent of a control pool.

Results of haematology and coagulation parameters before and after referral are summarised in Table 1. A serum biochemistry profile was normal. Due to suspicion of ongoing intracranial haemorrhage, one unit of fresh whole blood was administered as a supplementary source of VWF. Thoracic radiographs showed a mild, diffuse interstitial pattern. One day after admission, a menace response was present in the animal's right eye. Thoracic radiography was repeated the following day due to persistent tachypnoea and panting. A marked interstitial pattern with a bronchial component in the caudal lung fields and generalised cardiomegaly were present (Fig 2). Tracheal lavage yielded haemorrhagic fluid containing frequent clumps of metastrongyle larvae consistent in appearance with *A vasorum* (Fig 3). A Baermann test was also subsequently found to be positive for *A vasorum* larvae. Drug therapy was commenced as follows: 50 mg/kg fenbendazole (Panacur; Hoechst) administered orally every 24 hours for 21 days to treat angiostrongylosis; 250 mg amoxycillin-clavulanate (Synulox; Pfizer) every 12 hours for seven days as a prophylaxis

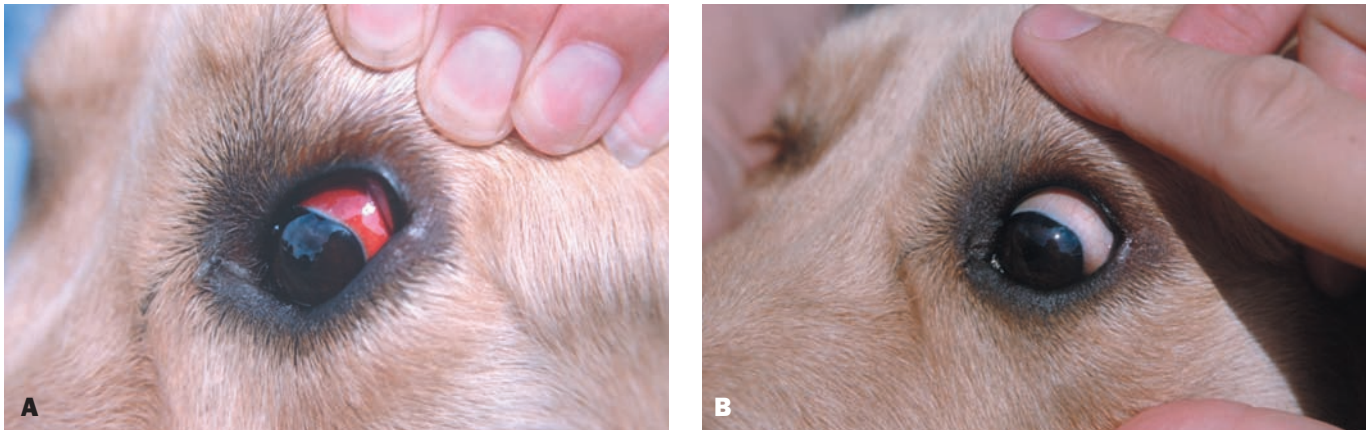

FIG 1. (A) Conjunctival haemorrhage obscures the sclera at the time of referral. (B) Conjunctival haemorrhage near resolution 14 days later

Table 1. Haematology and coagulation parameters

	Days after referral							
	–2	0 (pre-DDAVP)	0 (post-DDAVP)	2	10	28	655	Normal values
Haematocrit (litre/litre)	39·4		40					37-55
Haemoglobin (g/litre)	13							12-18
MCHC (%)	33							30-36·9
WBC ($\times 10^9$/litre)	9·3		14·7					6-16·9
Neutrophils ($\times 10^9$/litre)	6·8		12·35					2·8-10·5
Eosinophils ($\times 10^9$/litre)	0·6							0·5-1·5
Lymphocytes/monocytes ($\times 10^9$/litre)	1·9							1·1-6·3
Platelets ($\times 10^9$/litre)	164		157				319	155-450
OSPT (seconds)	11		15·2				8	6-11
APTT (seconds)	18·3		18·9				13·1	10-20
Fibrinogen (g/litre)	<0·5		1·0				1·5	1-4
FDP	Positive		Negative				Negative	0
BMBT (minutes)		>30	3	2	2			<4
VWF antigen (% of control pool)		22	61			66	92	70-180

DDAVP Desmopressin, MCHC Mean cell haemoglobin concentration, WBC Total white blood cell count, OSPT One-stage prothrombin time, APTT Activated partial thromboplastin time, FDP Fibrin degradation products, BMBT Buccal mucosal bleeding time, VWF von Willebrand factor

against opportunistic pneumonia; and 0·1 mg/kg dexamethasone administered subcutaneously every 24 hours for three days, for pneumonitis and prophylaxis against anaphylaxis caused by the death and embolisation of mature and immature stages of *A vasorum*.

Urinalysis performed four days after admission showed no evidence of proteinuria, which might have suggested concurrent glomerulonephritis. Eight days after admission, respiratory signs were much improved, although a moderate bronchointerstitial pattern remained. A magnetic resonance imaging (MRI) scan of the brain showed an expansile, space-occupying lesion (1·8 cm in diameter) of mixed

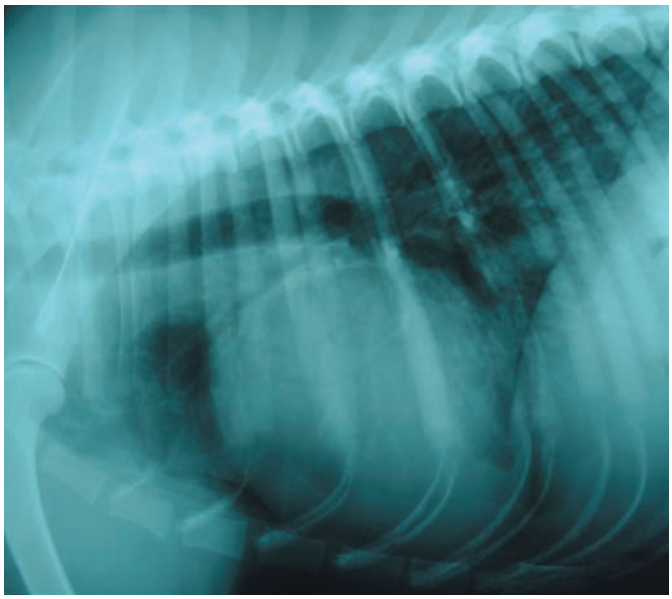

FIG 2. Right lateral radiograph of the thorax showing caudal (dorsal and ventral) bronchointerstitial pattern and generalised cardiomegaly

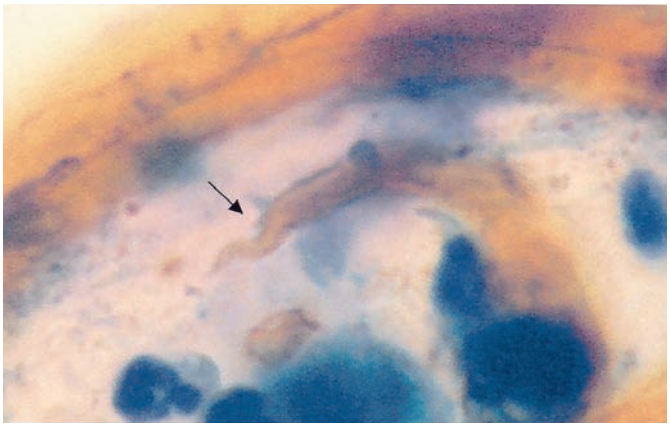

FIG 3. Haemorrhagic tracheal lavage fluid showing the tail of a nematode larva bearing a prominent dorsal notch (arrow), which helped to identify it as *Angiostrongylus vasorum*. Papanicolaou stain. ×1280

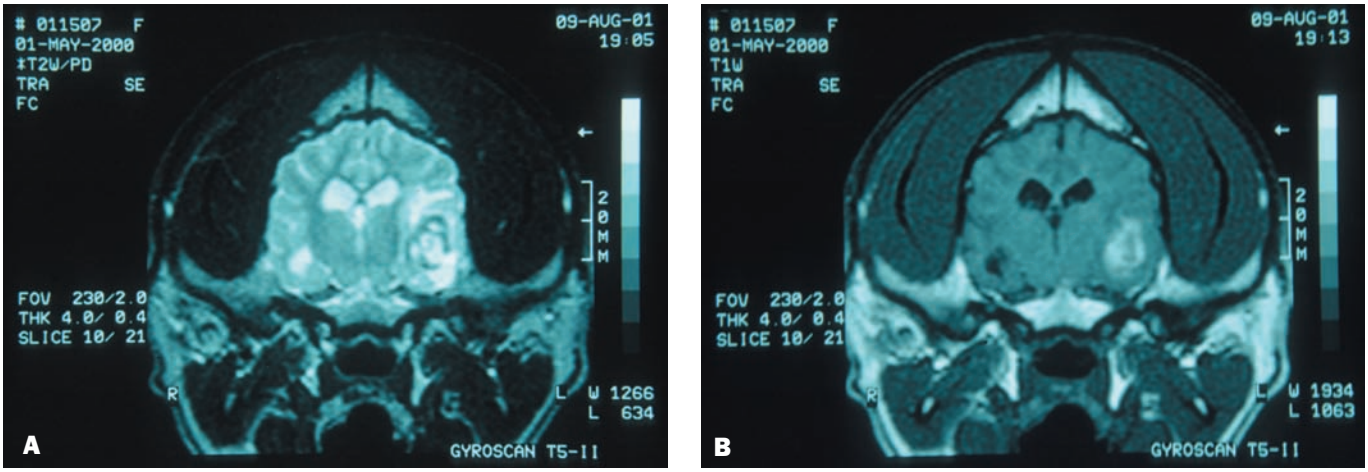

FIG 4. (A) T2-weighted transverse section magnetic resonance image of the brain showing the left-sided 1·8 cm space-occupying lesion of mixed signal intensity. (B) T1-weighted transverse section showing the same lesion. The periphery of the lesion is of low signal intensity on the T1-weighted image. This may represent the presence of intracellular methaemoglobin in an organising haematoma. This is surrounded by an ill-defined area of high signal intensity on the T2-weighted image, which is of low signal intensity on the T1-weighted image, suggesting brain oedema

signal intensity on the left, in the region of the hippocampus. An ill-structured area of high signal intensity on a T2-weighted image (Fig 4a) and low signal intensity on a T1-weighted image (Fig 4b) surrounded the lesion, suggesting a small degree of brain oedema. There was no contrast enhancement after the intravenous injection of the paramagnetic contrast agent gadoteridol (Prohance; Merck) at a dose of 0·1 mmol/kg. The following day BMBT remained normal, conjunctival haemorrhage was nearly resolved (Fig 1b) and the dog was discharged. Two weeks later the dog was clinically normal and the VWF level was 66 per cent. On follow-up at days 14 and 655 after initial referral, the dog was clinically normal. On day 655, the VWF level was 92 per cent and other coagulation parameters were also normal.

DISCUSSION

The appearance on MRI scans of a well-circumscribed focal lesion of mixed signal intensity was consistent with an organising haematoma containing varying amounts of paramagnetic material (methaemoglobin). Granulomatous inflammation may also have contributed to this picture. The sudden deterioration in clinical signs and the transient neurological deficits eight days previously suggested formation or rapid expansion of the space-occupying lesion at this time, consistent with intracranial haemorrhage due to coagulopathy or aberrant parasite migration. Perry and others (1991) reported granulomatous inflammation, gliosis and haemorrhage centred around capillaries containing larvae throughout the brain in a case of angiostrongylosis with disseminated larval infection causing signs of ocular and nervous disease, but coagulation results were not reported.

Haemorrhage at sites distant from the abdominal and thoracic viscera that constitute normal parts of the *Angiostrongylus* life cycle is well recognised. However, it is not clear whether aberrant parasite migration plays a role in some or all of these cases. A recent report documents sublingual haemorrhage (Ribiere and others 2001) and reports from an area of high prevalence suggest that conjunctival and sublingual haemorrhage are common (S. Manning, personal communication). A propensity for haemorrhage to occur in these locations might indicate aberrant larval migration, with the sites of haemorrhage representing predilection sites for aberrant migration and, hence, local vessel trauma. It is not known whether aberrant parasite migration is associated with more severe coagulopathies than 'conventional' angiostrongylosis.

First-stage (L1) *Angiostrongylus* larvae are microscopic and would not have been visible on the MRI images in this report. Adult worms measure 14 to 16 mm (Bolt and others 1994) and have been found in the anterior chamber of dogs with angiostrongylosis (King and others 1994), but not the subconjunctival tissue. Although in theory any adult worms present in the eyes or brain of the animal reported here might have been visible on MRI, the chances of a section of the worm sufficiently long to be discernible being parallel to the plane of the image were slim. Pathogenesis and previous reports of aberrant larval migration and embolisation are discussed by Bolt and others (1994).

The role of chronic disseminated intravascular coagulation (DIC) in canine angiostrongylosis has been demonstrated in a clinical case and reviewed (Ramsey and others 1996). This is further supported by findings in experimentally infected dogs (Prestwood and others 1981, Schelling and others 1986, Cury and others 2002b). Immune-mediated thrombocytopenia in angiostrongylosis is also documented (Gould and McInnes 1999). However, previously reported cases have not recorded abnormal primary haemostasis (BMBT) or VWF levels. It is possible that some degree of DIC was present in the case described here, supported by the presence of fibrin degradation products and low fibrinogen documented on one occasion pre-referral and the mild prolongation of OSPT recorded from a sample drawn after desmopressin administration. It is notable that the dog was not thrombocytopenic, as thrombocytopenia is frequently a component of DIC. However, a defect in primary haemostasis was considered likely due to occurrence of haemorrhage in the presence of a normal platelet count, OSPT and APTT documented prior to referral.

Correction of the BMBT to normal after administration of desmopressin was strongly suggestive of a lack of functional VWF (Kraus and others 1989). This was subsequently confirmed when very low VWF levels were reported (22 per cent). One month after treatment, VWF levels were 66 per cent. In screening healthy animals

for breeding purposes, levels from 50 to 69 per cent of a standard control are considered borderline (either normal or a heterozygous carrier of a gene for von Willebrand disease) and levels of 70 to 180 per cent are considered normal. Documentation of VWF levels of 92 per cent on follow-up two years after initial presentation strongly suggested that the previous low levels resulted from an acquired disorder rather than genetic influences.

Consumptive coagulopathy triggered by extensive endarteritis and possibly aberrant larval migration may have been the cause of the low VWF levels. Why this factor should have been depleted so markedly in this case when only minor derangements in other clotting parameters occurred is not apparent. Although DIC is often described as a consumptive coagulopathy, VWF levels are rarely evaluated when DIC is suspected and information on VWF levels in DIC in veterinary patients is lacking. However, a study on 346 human patients with DIC found that VWF was elevated in 80 per cent of patients evaluated (Spero and others 1980) and current medical texts report VWF levels to be variable in DIC, but most often normal or increased, due to release from endothelial storage sites (Provan and others 1998, Grosset and Rodgers 1999).

Therefore, the authors suggest that the case in the present report resembles the human condition of acquired von Willebrand syndrome. This is a rare bleeding disorder with laboratory findings similar to those of congenital von Willebrand disease. Cases are associated with lymphoproliferative, myeloproliferative, neoplastic, immunological, cardiovascular and other miscellaneous disorders. Proposed mechanisms for accelerated removal of VWF from the plasma include: specific antibodies to factor VIII/VWF; non-specific antibodies forming complexes with VWF, cleared by Fc-receptor bearing cells; absorption of VWF onto malignant cells; increased proteolytic degradation of VWF; and loss of large VWF multimers in high sheer stress conditions. None of the proposed mechanisms are specific for the different underlying disorders (Federici and others 2000). Although speculative, since canine angiostrongylosis has been associated with immune-mediated thrombocytopenia (Gould and McInnes 1999), other immune derangements, including antibody formation to VWF, might occur. Furthermore, a heavy burden of *Angiostrongylus* might be expected to cause high sheer stress conditions, as occurs with the other important canine intravascular parasite, *Dirofilaria immitis*.

Although the radiographic findings and coagulopathy documented in this case were strongly suggestive of *A vasorum*, definitive identification was provided by measurement of size (>320 to 340 µm, n=3) and the shape of the tail which featured a prominent dorsal notch (Fig 3), consistent with the comprehensive description by Guilhon and Cens (1973), and distinguishing the nematode from *Filaroides* species and other lungworms of dogs (Pinckney 2000).

The increasing prevalence of this parasite, the potential for shedding large numbers of larvae by asymptomatic dogs, carriage by foxes and difficulties in preventing ongoing access of dogs to mollusc intermediate hosts have implications for parasite control and prophylaxis. Detection of occult infections will only improve with more routine use of the Baermann technique for detecting larvae in faeces. A reduction in both prevalence of infection and morbidity in dogs would be expected with more routine inclusion of an agent active against *Angiostrongylus* (such as fenbendazole) in roundworm prophylaxis regimens.

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References

Bolt, G., Monrad, J., Koch, J. & Jensen, A. L. (1994) Canine angiostrongylosis: a review. *Veterinary Record* **135**, 447-452

Cury, M. C., Guimaraes, M. P., Lima, W. S. & Vitor, R. W. (2002a) Western blot analysis of the humoral response of dogs experimentally infected with *Angiostrongylus vasorum* (Baillet, 1866). *Veterinary Parasitology* **106**, 83-87

Cury, M. C., Lima, W. S., Guimaraes, M. P. & Carvalho, M. G. (2002b) Hematological and coagulation profiles in dogs experimentally infected with *Angiostrongylus vasorum* (Baillet, 1866). *Veterinary Parasitology* **104**, 139-149

Federici, A. B., Rand, J. H., Bucciarelli, P., Budde, U., van Genderen, P. J., Mohri, H., Meyer, D., Rodeghiero, F. & Sadler, J. E. (2000) Acquired von Willebrand syndrome: data from an international registry. *Thrombosis and Haemostasis* **84**, 345-349

Gould, S. M. & McInnes, E. L. (1999) Immune-mediated thrombocytopenia associated with *Angiostrongylus vasorum* infection in a dog. *Journal of Small Animal Practice* **40**, 227-232

Grosset, A. B. M. & Rodgers, G. M. (1999) Acquired coagulation disorders. In: Wintrobe's Clinical Haematology. 10th edn. Blackwell publishing, Oxford. p 1736

Guilhon, J. & Cens, B. (1973) *Angiostrongylus vasorum* (Baillet, 1866). Morphology and biological study. *Annales de Parasitologie Humaine et Comparee* **48**, 567-595

King, M. C. A., Grose, R. M. R. & Startup, G. (1994) *Angiostrongylus vasorum* in the anterior chamber of a dog's eye. *Journal of Small Animal Practice* **35**, 326-328

Kraus, K. H., Turrentine, M. A., Jergens, A. E. & Johnson, G. S. (1989) Effect of desmopressin acetate on bleeding times and plasma von Willebrand factor in Doberman pinscher dogs with von Willebrand's disease. *Veterinary Surgery* **18**, 103-109

Perry, A. W., Hertling, R. & Kennedy, M. J. (1991) Angiostrongylus with disseminated larval infection associated with signs of ocular and nervous disease in an imported dog. *Canadian Veterinary Journal* **32**, 430-431

Pinckney, R. D. (2000) Canine *filaroides* infection. In: Companion and Exotic Animal Parasitology. Ed D. D. Bowman. International Veterinary Information Service, Ithaca. www.ivis.org

Prestwood, A. K., Greene, C. E., Mahaffey, E. A. & Burgess, D. E. (1981) Experimental canine angiostrongylosis: I. Pathologic manifestations. *Journal of the American Animal Hospital Association* **17**, 491-497

Provan, D., Chisholm, M., Duncombe, A. S., Singer, C. R. J. & Smith, A. G. (1998) Haematological emergencies. In: Oxford Handbook of Clinical Haematology. Oxford University Press, Oxford. p 423

Ramsey, I. K., Littlewood, J. D., Dunn, J. K. & Herrtage, M. E. (1996) Role of chronic disseminated intravascular coagulation in a case of canine angiostrongylosis. *Veterinary Record* **138**, 360-363

Ribiere, T., Gottstein, B., Huber, E., Welle, M., Forster, J. L. & Grosclaude, P. (2001) A case of canine angiostrongylosis. *Schweizer Archiv fur Tierheilkunde* **143**, 313-318

Schelling, C. G., Greene, C. E., Prestwood, A. K. & Tsang, V. C. (1986) Coagulation abnormalities associated with acute *Angiostrongylus vasorum* infection in dogs. *American Journal of Veterinary Research* **47**, 2669-2673

Spero, J. A., Lewis, J. H. & Hasiba, U. (1980) Disseminated intravascular coagulation. Findings in 346 patients. *Thrombosis and Haemostasis* **43**, 28-33

Hypercalcaemia associated with *Angiostrongylus vasorum* in three dogs

Hypercalcaemia was identified in three dogs that presented primarily for evaluation of respiratory disease. Angiostrongylosis was diagnosed in all three cases and both the respiratory signs and the hypercalcaemia resolved with treatment. Infection with *Angiostrongylus vasorum* is known to lead to formation of pulmonary granulomata. Granulomatous disease in humans may lead to hypercalcaemia secondary to increased unregulated production of 1,25-dihydroxycholecalciferol by activated macrophages in the granulomata. In one of the three dogs, 1,25-dihydroxycholecalciferol was measured and found to be increased, providing supportive evidence for a similar mechanism in dogs. To the authors' knowledge, hypercalcaemia has not previously been reported in association with angiostrongylosis in dogs. Since prolonged untreated hypercalcaemia may lead to permanent impairment of renal function, dogs with angiostrongylosis should be evaluated for the presence of hypercalcaemia.

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INTRODUCTION

Hypercalcaemia occurs relatively uncommonly in dogs (Chew and Meuten 1982) and is associated with a spectrum of non-specific clinical signs such as polyuria/polydipsia, anorexia and weight loss (Chew and Carothers 1989). Since hypercalcaemia can lead to severe renal, gastrointestinal, cardiovascular and neurological dysfunction (Kruger and others 1996), its identification should prompt assessment of the patient for an underlying cause. If the aetiology of the hypercalcaemia is not immediately evident, then symptomatic therapy can be initiated until a diagnosis is reached and specific therapy started.

Commonly reported causes of hypercalcaemia in dogs include hypercalcaemia of malignancy (frequently associated with lymphoma and anal sac apocrine gland adenocarcinoma), hypoadrenocorticism, renal failure, primary hyperparathyroidism, hypervitaminosis D, non-malignant skeletal lesions and non-pathological causes, such as young growing animals, laboratory errors and lipaemia (Martin 1998).

Hypercalcaemia has also been reported in association with granulomatous disease in dogs (Dow and others 1986, Troy and others 1986, Arceneaux and others 1998, Barrett and others 1998, Rohrer and others 2000, Fradkin and others 2001) and cats (Hodges and others 1994, Mealey and others 1999). This association is well recognised in human medicine where the abnormalities of calcium metabolism are due to dysregulation of 1,25-dihydroxycholecalciferol (calcitriol) production by activated macrophages (Hewison and others 2000, Sharma 2000).

Angiostrongylus vasorum is a metastrongylid nematode that infects domestic dogs and related canids. The nematode has an indirect life cycle using gastropods (slugs and snails) as an intermediate host. The adult worms reside in the right heart and pulmonary vasculature, with the adults laying eggs in terminal pulmonary arterioles. The eggs hatch and the first-stage larvae, which migrate through alveolar walls, are then coughed up, swallowed and subsequently excreted in the faeces (Rosen and others 1970).

Clinical signs commonly reported include cough, dyspnoea, collapse and bleeding diatheses (Martin and others 1993, Patteson and others 1993, Bolt and others 1994). Histopathologically, infection with *A vasorum* is characterised by the development of multifocal coalescing granulomas in the periphery of the lungs accompanied by haemorrhage, arterial thromboses and periarteritis (Prestwood and others 1981).

This report describes three dogs with confirmed angiostrongylosis, hypercalcaemia identified on an initial serum biochemical panel and clinical signs consistent with hypercalcaemia. No other cause for the hypercalcaemia was identified and in all cases it resolved following treatment of the parasite.

CASE HISTORIES

Case 1

An eight-year-old, neutered female golden retriever was presented to the Queen Mother Hospital, Royal Veterinary College, for evaluation of dyspnoea, cough, inappetence and weight loss of two months' duration. Physical examination revealed an elevated respiratory rate (40 breaths/ minute) and increased respiratory effort. On thoracic auscultation, crackles were audible bilaterally especially over the dorsocaudal lung fields. The rest of the physical examination was unremarkable. Routine haematology (Table 1), serum biochemistry, and electrolyte and venous blood gas analysis (Table 2) were performed. Free catch urinalysis was unremarkable. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were mildly prolonged compared with control samples (PT 11·9 seconds, control 10·5 seconds; APTT 22·9 seconds, control 20·2 seconds).

Thoracic radiography revealed a mixed interstitial/alveolar pattern with mainly peripheral distribution. Bronchoscopy revealed increased mucus in the airways and cytological examination of bronchoalveolar lavage (BAL) samples showed low numbers of *A vasorum* larvae accompanied by suppurative inflammation. Bacterial culture of the BAL was negative. Baermann examination of a faecal sample confirmed the presence of *A vasorum* larvae. Treatment with fenbendazole (Panacur; Hoechst) was initiated at a dose of 50 mg/kg once daily. Saline diuresis was started with 4 ml/kg 0·9 per cent sodium chloride per hour intravenously to treat the hypercalcaemia.

Diagnostic tests were pursued to identify a cause for the hypercalcaemia. Ultrasound examinations of the abdomen and parathyroid gland region were unremarkable. An adrenocorticotrophic hormone stimulation test was not consistent with hypoadrenocorticism. Bone marrow cytology showed mild megakaryocytic hyperplasia but no evidence of neoplasia. Parathyroid hormone (PTH) and PTH related peptide (PTHrP) concentrations were within normal references ranges (Table 3). Hypervitaminosis D was considered an unlikely cause of the hypercalcaemia since there was no history of toxin ingestion and serum phosphorus concentration was within reference ranges. Following 24 hours of saline diuresis, the ionised calcium concentration was still elevated (1·65 mmol/litre) and 1 mg/kg frusemide (Dimazon; Intervet) intravenously three times a day was added to the treatment regimen for its calciuretic effect.

By day 4, the dog was more dyspnoeic and nasal oxygen therapy was introduced. It was considered that the dyspnoea might have worsened with parasite death, which could have incited an inflammatory response. The ionised calcium was still increased at 1·65 mmol/litre. Dexamethasone (Dexadreson; Intervet) therapy was initiated at a dose of 0·1 mg/kg intravenously once daily to treat both the hypercalcaemia and airway inflammation.

By day 7, the ionised calcium was within the reference interval (1·44 mmol/litre); however, the dog was still dyspnoeic. Repeat thoracic radiographs were essentially unchanged. A repeat endotracheal wash was performed which revealed continued verminous inflammation. As the accompanying inflammatory cell population now consisted of degenerate neutrophils, a secondary bacterial pneumonia was suspected. Intravenous amoxycillin/clavulanate (Augmentin; Glaxo Smithkline) was initiated at a dose of 20 mg/kg three times daily. On day 9, culture results confirmed bacterial pneumonia with growth of a *Pasteurella* species which was sensitive to amoxycillin/clavulanate.

On days 10 to 13, the dog started to show a significant improvement in

Table 1. Haematological results (reference ranges) at initial evaluation. Abnormal results are shown in bold

	Case 1	Case 2	Case 3
Haematocrit (%)	46·1 (37-55)	42·3 (37-55)	44·9 (35-55)
Neutrophils ($\times 10^9$/litre)	6·66 (3-11·5)	**14·48** (3-11·5)	9·42 (2·5-9)
Eosinophils ($\times 10^9$/litre)	**2·78** (0-1·3)	**1·88** (0-1·3)	**1·67** (0·1-1·0)
Lymphocytes ($\times 10^9$/litre)	1·33 (1-4·8)	**0·75** (1-4·8)	3·19 (1·5-4)
Monocytes ($\times 10^9$/litre)	1·33 (0·15-1·5)	**1·69** (0·15-1·5)	0·61 (0·1-0·8)
Basophils ($\times 10^9$/litre)	0 (0)	0 (0)	0·15 (rare)
Platelets ($\times 10^9$/litre)	**96·7** (150-900)	**98** (150-900)	**129** (200-400)

Reference ranges varied depending on the laboratory used

Table 2. Biochemical and venous blood gas results (reference ranges) at initial evaluation. Abnormal results are shown in bold

	Case 1	Case 2	Case 3
Albumin (g/litre)	33·8 (25-39)	30·6 (25-39)	**35·3** (36-44)
Globulin (g/litre)	40·8 (27-44)	**48·1** (27-44)	**70·5** (20-35)
Total calcium (mmol/litre)	**3·09** (2·2-2·85)	**3·35** (2·2-2·85)	**3·60** (2·15-2·5)
Ionised calcium (mmol/litre)	**1·67** (1-1·5)	**1·73** (1-1·5)	Not done
pH	7·42 (7·35-7·45)	7·41 (7·35-7·45)	Not done
Urea (mmol/litre)	**12·9** (3-10)	3·4 (3-10)	5·9 (2-7)
Creatinine (µmol/litre)	126 (50-140)	106 (50-140)	**68** (70-110)
Phosphorus (mmol/litre)	1·59 (1-2·52)	1·93 (1-2·52)	1·53 (0·75-1·25)
Alanine aminotransferase (U/litre)	31 (22-220)	31 (22-220)	23 (20-60)
Alkaline phosphatase (U/litre)	132 (30-250)	103 (30-250)	46 (<110)
Sodium (mmol/litre)	147 (145-155)	153 (145-155)	Not done
Potassium (mmol/litre)	3·7 (3·5-5·5)	4·3 (3·5-5·5)	Not done

Reference ranges varied depending on the laboratory used

Table 3. Ionised calcium, parathyroid hormone (PTH), parathyroid hormone-related peptide (PTHrP) and vitamin D metabolite levels. Abnormal results are shown in bold

	Case 1	Case 2	Reference ranges
PTH (pg/ml)	**<5·0**	**9·3**	10-60
PTHrP (pg/ml)	0·4	0·3	<0·5
25-hydroxycholecalciferol (ng/ml)	Not done	**10·9**	19·3-43·6*
1,25-dihydroxycholecalciferol (pg/ml)	Not done	**54**	16-38*

*Reference range generated from 11 normal dogs (Mellanby and others 2003)

demeanour and respiratory character. Ionised calcium was normal throughout this time. Nasal oxygen, intravenous fluid and intravenous drug therapies were discontinued on day 12. Oral amoxycillin/clavulanate (Synulox; Pfizer) at a dose of 20 mg/kg twice daily and 0·5 mg/kg oral prednisolone (Prednidale; Arnolds) once daily were instigated and oral fenbendazole was continued. Thoracic radiographs on day 13 showed marked reduction in the severity of the pulmonary pattern and repeat faecal examination was negative for *A vasorum*. The dog was discharged on day 15 with a further seven day course of fenbendazole and oral amoxycillin/clavulanate. Prednisolone dosage was reduced to 0·5 mg/kg every other day.

The dog was re-examined on day 28 when the owner reported that it had been well at home. Physical examination revealed a mildly increased respiratory rate with normal respiratory effort. Haematology and serum biochemistry were unremarkable with normal total and ionised calcium concentrations (2·49 mmol/litre and 1·32 mmol/litre, respectively). Thoracic radiographs showed resolution of the alveolar pattern. A mild peribronchial/interstitial pattern was present in the periphery of the lung field. Baermann faecal examination was negative for *A vasorum* larvae. All medications were discontinued.

During a further examination on day 61, the owner reported that the dog was still acting normal at home. Physical examination was unremarkable and the dog had gained weight. Baermann faecal examination was negative and ionised calcium was normal at 1·3 mmol/litre. Telephone conversation with the owner 18 months after discharge revealed the dog to be normal with no further problems.

Case 2

An 11-month-old, entire male bulldog was presented to the Queen Mother Hospital, Royal Veterinary College, for evaluation of cough, dyspnoea, inappetence and weight loss of one month's duration and marked polyuria/polydipsia of one week's duration. On presentation, the animal was receiving 12·5 mg/kg oral amoxycillin/clavulanate twice daily. On physical examination, the dog was underweight and markedly dyspnoeic with increased respiratory effort and a respiratory rate of 68 breaths/minute. Auscultation of the lungs revealed crackles on deep inspiration and referred upper respiratory tract noise. No other abnormalities were found.

Routine haematology (Table 1), serum biochemistry, and electrolyte and venous blood gas analysis (Table 2) were performed. PT and APTT, measured by an in-house bench-top analyser (SCA 2000; Synbiotics), were within normal limits.

Thoracic radiography revealed a marked alveolar pattern with air bronchograms principally in the periphery of the lung fields. Centrally, the lung fields demonstrated an interstitial pattern. Bronchoscopy revealed bloody mucus in all airways. Cytological examination of BAL samples revealed numerous *A vasorum* larvae and a mixed population of inflammatory cells (73 per cent neutrophils, 4 per cent lymphocytes, 22 per cent macrophages and 1 per cent eosinophils). Bacterial culture was negative. A faecal sample could not be obtained at this time. Abdominal ultrasound was unremarkable. Samples for measurement of PTH, PTHrP and vitamin D metabolites were submitted (Table 3).

Treatment was initiated with 5 ml/kg 0·9 per cent sodium chloride solution per hour intravenously, 50 mg/kg fenbendazole once daily, 0·1 mg/kg dexamethasone intravenously once daily and 10 mg/kg enrofloxacin (Baytril; Bayer) intravenously once daily to treat possible concurrent bacterial pneumonia. The dog made a slow but steady improvement in his demeanour, appetite and respiratory character. Ionised calcium levels were monitored daily and gradually returned to within the reference interval (1·46 mmol/litre on day 4). Intravenous fluid and drug therapies were discontinued on day 8. Oral prednisolone therapy at a dose of 0·25 mg/kg twice daily was initiated and fenbendazole was continued. The dog was discharged on day 10. A faecal sample taken at this time was negative for *A vasorum* larvae.

The dog was re-examined on day 16. The owners reported the dog to be well, with a good appetite and no polydipsia. Although still underweight, there was some weight gain. Respiratory rate and effort were normal and thoracic auscultation revealed referred upper respiratory tract noises typical of the breed. Baermann faecal analysis was negative. Ionised calcium was within the reference interval at 1·42 mmol/litre. Fenbendazole was discontinued and the owners were instructed to reduce prednisolone to 0·25 mg/kg once daily for seven days, then to 0·25 mg/kg every other day for four doses.

During re-examination on day 40, the dog was reported to be well at home. Physical examination revealed some weight gain and upper airway abnormalities typical of the breed. Thoracic radiographs revealed a marked improvement in the

appearance of the lung fields with only a mild residual bronchointerstitial pattern in the caudodorsal lung fields. Baermann faecal examination was negative and the ionised calcium concentration was 1·4 mmol/litre. Telephone follow-up with the owners on day 60 revealed that the dog was clinically normal.

Case 3

A 10-month-old, entire male Staffordshire bull terrier was presented to the Veterinary Hospital of the University of Bristol for evaluation of a two-month history of cough and recurrent episodes of depression. Empirical antibiotic and corticosteroid therapy was initiated by the referring veterinary surgeon resulting in some clinical response. The dog then scratched his cornea and corticosteroid therapy was discontinued. At this point the dog deteriorated with progressive dyspnoea, weight loss and inappetence.

Physical examination revealed a depressed and underweight dog. Respiratory rate (74 breaths/minute) and effort were markedly increased, and thoracic auscultation revealed increased respiratory sounds over all lung fields. Ophthalmic examination revealed congested conjunctival vessels suggestive of hyperviscosity while fundic examination revealed granulomatous lesions and chorioretinitis. The corneal scratch healed well. No other abnormalities were found.

Routine haematology (Table 1) and serum biochemistry (Table 2) were performed. Serum protein electrophoresis showed that the hyperglobulinaemia was due to elevations of the beta- and gamma-globulins. PT and APTT were within normal limits. Urinalysis was unremarkable.

Thoracic radiography showed large multifocal, nodular, soft tissue opacities in the caudodorsal and middle lung fields with air bronchograms visible in the caudodorsal lung field. Abdominal radiographs and echocardiography were unremarkable. A faecal sample examined by Baermann flotation was positive for *A vasorum* larvae.

Therapy was initiated with 4 ml/kg 0·9 per cent sodium chloride solution every hour; 50 mg/kg fenbendazole once daily for 15 days; 0·25 mg/kg dexamethasone subcutaneously once on day 1 as an anti-inflammatory; 10 mg/kg oral amoxycillin/clavulanate twice daily for seven days to treat possible secondary bacterial pneumonia; and 2 mg/kg frusemide subcutaneously twice daily to aid with calciuresis.

The dog's condition improved steadily. By day 6, serum total calcium had decreased to 2·8 mmol/litre. The frusemide dose was reduced to 1 mg/kg twice daily for one day and then discontinued. On day 7, thoracic radiographs revealed some resolution of the nodular lesions. Intravenous fluid therapy was then discontinued and the dog was discharged exhibiting mild tachypnoea as its only clinical sign. Total calcium at discharge was slightly above the reference interval at 2·68 mmol/litre and ionised calcium was 1·36 mmol/litre

The dog was re-examined on day 60. The owners reported that the dog was normal at home. Physical examination was unremarkable. Faecal analysis was negative for *A vasorum* larvae and thoracic radiographs showed only residual bronchointerstitial markings. Haematology and biochemistry were within reference intervals.

DISCUSSION

The three dogs included in this report presented primarily for investigation of respiratory disease. In all three cases, a diagnosis of angiostrongylosis was confirmed by the identification of *A vasorum* first-stage larvae either in BAL fluid, faeces or both. The three dogs exhibited many of the clinical signs (cough, exercise intolerance and dyspnoea) and clinicopathological abnormalities (eosinophilia, mild thrombocytopenia and hyperglobulinaemia) described in previous case series of angiostrongylosis (Martin and others 1993, Patteson and others 1993, Chapman and others 2004). Thoracic radiographic findings were also similar to those reported previously (Martin and others 1993, Patteson and others 1993, Boag and others 2004). All three dogs ultimately responded well to treatment with fenbendazole, which is the recommended treatment of this disease (Bolt and others 1994), with resolution of their respiratory signs. In all three cases, hypercalcaemia was documented on the initial serum biochemistry panel.

Regulation of serum calcium concentration is complex and involves the integrated actions of several hormones, notably PTH and vitamin D and its metabolites (Ganong 2001). Clinical detection of hypercalcaemia is not always easy because it is the total serum calcium (consisting of protein-bound, complexed and ionised fractions) that is most commonly measured, although it is the ionised fraction that is metabolically active and which, if increased or decreased, results in clinical signs (Schenck and others 1996). Non-pathological hypercalcaemia may be associated with haemoconcentration, hyperproteinaemia and analytical interference (for example, lipaemia).

All three dogs in this report demonstrated one or more clinical signs associated with hypercalcaemia (for example, inappetence, weight loss, polyuria/polydipsia and lethargy) as well as respiratory signs. In two cases, a significant increase in ionised calcium as well as total calcium was confirmed. Two of the animals were less than one year of age and calcium levels are slightly increased in young growing animals (Mischke and others 1996). However, the presence of clinical signs and the magnitude of the hypercalcaemia make it very likely that the hypercalcaemia was pathological in all three cases.

Whenever pathological hypercalcaemia is identified, a thorough search for the underlying disease process is warranted. At the time of examination of these cases, hypercalcaemia had not been reported in association with angiostrongylosis. A subsequent retrospective review, including one of the cases described more fully here (case 1), identified a mild to moderately elevated

total calcium in three of 23 cases of angiostrongylosis (Chapman and others 2004). The golden retriever (case 1) was significantly older than the majority of dogs with angiostrongylosis (Martin and others 1993, Patteson and others 1993, Chapman and others 2004) leading to a high index of suspicion for concurrent neoplasia. A thorough evaluation for other causes of hypercalcaemia was performed in this case. None were identified. The low PTH level was consistent with suppression of the parathyroid glands due to the hypercalcaemia.

Corticosteroids were administered in all three cases, aiming to reduce the inflammatory reaction in the pulmonary tissue that may accompany death of the nematode (Bolt and others 1994). Corticosteroids tend to decrease serum calcium concentration by reducing bone resorption, decreasing intestinal calcium absorption and increasing renal calcium excretion (Mahgoub and others 1997). Corticosteroids are also lymphocytolytic and, in animals with hypercalcaemia secondary to lymphoma, their administration can lead to a rapid reduction in serum calcium levels. However, the lymphocytolysis can make definitive histopathological diagnosis of lymphoma difficult or impossible (Rosol and others 2000). It is possible that the administration of corticosteroids in the dogs in the present case masked an alternative cause for their hypercalcaemia such as lymphoma. However, all three cases were clinically well with normal serum calcium levels several months after withdrawal of corticosteroid therapy. It is highly unlikely that the use of corticosteroids masked other underlying disease in any of the patients.

Hypercalcaemia has been associated with other granulomatous diseases in dogs including blastomycosis (Dow and others 1986, Arceneaux and others 1998), schistosomiasis (Troy and others 1986, Rohrer and others 2000, Fradkin and others 2001), pyogranulomatous dermatitis and panniculitis (Barrett and others 1998), retained foetus and endometritis (Hirt and others 2000) and granulomatous lymphadenitis (Mellanby and others 2003). The proposed mechanism is dysregulated production of 1,25-dihydroxycholecalciferol by activated macrophages (Dow and others 1986). It has been shown that mononuclear phagocytes, the cells most commonly involved in granulomatous disease, can metabolise 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol in vitro (Cohen and Gray 1984). Increased levels of 1,25-dihydroxycholecalciferol have been found in people with granulomatous disease of various aetiologies (Playford and others 2001, Sharma 2000) and have also been identified in a cat with granulomatous skin disease (Mealey and others 1999). To the authors' knowledge, 1,25-dihydroxycholecalciferol has only been measured in one of the previously reported canine cases (Mellanby and others 2003) where it was found to be elevated in a dog with granulomatous lymphadenitis. Levels of PTHrP were found to be elevated in two dogs with schistosomiasis (Fradkin and others 2001) but 1,25-dihydroxycholecalciferol was not measured in these patients.

In the present report, vitamin D metabolites were measured in case 2 and 1,25-dihydroxyvitamin D was found to be increased. PTH was found to be decreased and PTHrP was within the reference range. The concentration of 25-dihydroxycholecalciferol was below the reference interval indicating that it was unlikely that the elevated 1,25-dihydroxycholecalciferol level was due to unreported dietary supplementation or ingestion of toxins containing vitamin D or its analogues (Carothers and others 1994, Mellanby and others 2003). Interestingly, 25-dihydroxycholecalciferol was measured in several of the previously reported canine cases of hypercalcaemia (Barrett and others 1998, Rohrer and others 2000, Fradkin and others 2001) and found to be low in two cases (Barrett and others 1998, Fradkin and others 2001). The mechanism of this decrease is unclear, as formation of 25-hydroxycholecalciferol does not appear to be tightly regulated and largely depends on the amount of cholecalciferol presented to the liver as a substrate (Ganong 2001). Possible mechanisms could include decreased dietary intake of cholecalciferol, depletion of 25-hydroxycholecalciferol following increased conversion to 1,25-dihydroxycholecalciferol, or a negative feedback effect of either the ionised calcium or the 1,25-dihydroxycholecalciferol on production of 25-hydroxycholecalciferol.

Increases in 1,25-dihydroxycholecalciferol will tend to lead to increased serum phosphorus by enhancing intestinal phosphate absorption and it is interesting that serum phosphorus concentration was within reference intervals in case 2. It is possible that decreased dietary intake of phosphorus contributed to this, as the dog had been inappetent for several weeks. The phosphorus was mildly elevated in case 3 but vitamin D metabolite levels were not measured in this dog.

Prolonged hypercalcaemia may lead to permanent organ (especially renal) dysfunction following soft tissue mineralisation. In all of the cases presented here, the calcium-phosphorus product (with both values converted to mg/dl) was greater than 60 mg/dl leading to a high risk of soft tissue mineralisation (Meuten and others 1981, Morrow and Volmer 2002). Treatment using a combination of saline diuresis, frusemide and corticosteroid administration was used in all of the dogs to ameliorate the hypercalcaemia while a diagnosis was made and definitive anthelmintic therapy was instituted. All of the dogs made a full recovery with no evidence of permanent renal damage.

Hypercalcaemia appears to be a rare complication of angiostrongylosis. The aetiology is likely to be unregulated production of 1,25-dihydroxycholecalciferol by activated macrophages within pulmonary granulomata. Due to the potential for long term renal damage, calcium should be measured in all dogs with angiostrongylosis, especially if they are exhibiting concurrent clinical signs consistent with hypercalcaemia.

References

ARCENEAUX, K. A., TABOADA, J. & HOSGOOD, G. (1998) Blastomycosis in dogs: 115 cases (1980-1995). *Journal of the American Veterinary Medical Association* **213**, 658-664

BARRETT, S. J., SHEAFOR, S. E., HILLIER, A. & CHEW, D. J. (1998) Challenging cases in internal medicine: what's your diagnosis. *Veterinary Medicine* **93**, 35-44

BOAG, A. K., LAMB, C. R., CHAPMAN, P. S. & BOSWOOD, A. (2004) Radiographic findings in 16 dogs with *Angiostrongylus vasorum* infection. *Veterinary Record* **154**, 426-430

BOLT, G., MONRAD, J., KOCH, J. & JENSEN, A. L. (1994) Canine angiostrongylosis: a review. *Veterinary Record* **135**, 447-452

CAROTHERS, M. A., CHEW, D. J. & NAGODE, L. A. (1994) 25(OH)-cholecalciferol intoxication in dogs. Proceedings of the American College of Veterinary Internal Medicine Forum. San Francisco, USA, June 2 to 5, 1994. pp 822-825

CHAPMAN, P. S., BOAG, A. K., GUITIAN, J. & BOSWOOD, A. (2004) *Angiostrongylus vasorum* infection in 23 dogs (1999-2002). *Journal of Small Animal Practice* **45**, 435-440

CHEW, D. J. & CAROTHERS, M. (1989) Hypercalcemia. *Veterinary Clinics of North America* **19**, 265-287

CHEW, D. J. & MEUTEN, D. J. (1982) Disorders of calcium and phosphorus metabolism. *Veterinary Clinics of North America* **12**, 411-439

COHEN, M. S. & GRAY, T. K. (1984) Phagocytic cells metabolize 25-hydroxyvitamin D_3 in vitro. *Proceedings of the National Academy of Sciences USA* **81**, 931-934

DOW, S. W., LEGENDRE, A. M., STIFF, M. & GREENE, C. (1986) Hypercalcemia associated with blastomycosis in dogs. *Journal of the American Veterinary Medical Association* **188**, 706-709

FRADKIN, J. M., BRANIECKI, A. M., CRAIG, T. M., RAMIRO-IBANEZ, F., ROGERS, K. S. & ZORAN, D. L. (2001) Elevated parathyroid hormone-related peptide and hypercalcemia in two dogs with schistosomiasis. *Journal of the American Animal Hospital Association* **37**, 349-355

GANONG, W. F. (2001) Hormonal control of calcium metabolism and the physiology of bone. In: Review of Medical Physiology. 20th edn. Ed W. F. Ganong. McGraw-Hill, New York. pp 369-382

HEWISON, M., ZEHNDER, D., BLAND, R. & STEWART, P. M. (2000) 1α-hydroxylase and the action of vitamin D. *Journal of Molecular Endocrinology* **25**, 141-148

HIRT, R. A., KNEISSL, S. & TEINFALT, M. (2000) Severe hypercalcaemia in a dog with a retained fetus and endometritis. *Journal of the American Veterinary Medical Association* **216**, 1423-1425

HODGES, R. D., LEGENDRE, A. M., ADAMS, L. G., WILLARD, M. D., KITTS, R. P., MONCE, K., NEEDELS, C. C. & WARD, H. (1994) Itraconazole for the treatment of histoplasmosis in cats. *Journal of Veterinary Internal Medicine* **8**, 409-413

KRUGER, J. M., OSBORNE, C. A., NACHREINER, R. F. & REFSAL, K. R. (1996) Hypercalcemia and renal failure – etiology, pathophysiology, diagnosis and treatment. *Veterinary Clinics of North America* **26**, 1417-1445

MAHGOUB, A. M., HIRSCH, P. F. & MUNSON, P. L. (1997) Calcium-lowering action of glucocorticoids in adrenalectomised-parathyroidectomised rats: specificity and relative potency of natural and synthetic glucocorticoids. *Endocrine* **6**, 279-283

MARTIN, L. G. (1998) Hypercalcemia and hypermagnesemia. *Veterinary Clinics of North America* **28**, 565-585

MARTIN, M. W. S., ASHTON, G., SIMPSON, V. R. & NEAL, C. (1993) Angiostrongylosis in Cornwall: clinical presentations of eight cases. *Journal of Small Animal Practice* **34**, 20-25

MEALEY, K. L., WILLARD, M. D., NAGODE, L. A. & GAYMAN HELMAN, R. (1999) Hypercalcemia associated with granulomatous disease in a cat. *Journal of the American Veterinary Medical Association* **215**, 959-962

MELLANBY, R. J., FRIEND, E. J., MELLOR, P. J., HERRTAGE, M. E., MEE, A. & BERRY, J. (2003) Vitamin D metabolite concentrations in dogs with unusual calcium metabolism disorders. Proceedings of the European Society of Veterinary Internal Medicine. Uppsala, Sweden, September 4 to 6, 2003. p 178

MEUTEN, D. J., COOPER, B. J., CAPEN, C. C., CHEW, D. J. & KOCIBA, G. J. (1981) Hypercalcemia associated with an adenocarcinoma derived from the apocrine glands of the anal sac. *Veterinary Pathology* **18**, 454-471

MISCHKE, R., HANIES, R., LANGE, K. & RIVERA RAMINEZ, P. A. (1996) Einfluss der albuminkonzentration auf die Beziehung zwischen der Konzentration des ionisierten Calciums und Gesamtcalciums im Blut des Hundes. *Deutsche Tierarztliche Wochenschrift* **103**, 199-204

MORROW, C. K. & VOLMER, P. A. (2002) Hypercalcemia, hyperphosphataemia, and soft tissue mineralisation. *Compendium on Continuing Education for the Practicing Veterinarian* **5**, 380-387

PATTESON, M. W., GIBBS, C., WOTTON, P. R. & DAY, M. J. (1993) *Angiostrongylus vasorum* in seven dogs. *Veterinary Record* **133**, 565-570

PLAYFORD, E. G., BANSAL, A. S., LOOKE, D. F. M., WHITBY, M. & HOGAN, P. G. (2001) Hypercalcaemia and elevated 1,25 $(OH)_2D_3$ levels associated with disseminated *Mycobacterium avium* infection in AIDS. *Journal of Infection* **42**, 157-158

PRESTWOOD, A. K., GREENE, C. E., MAHAFFEY, E. A. & BURGESS, D. E. (1981) Experimental canine angiostrongylosis: I pathologic manifestations. *Journal of the American Animal Hospital Association* **17**, 491-497

ROHRER, C. R., PHILLIPS, L. A., FORD, S. L. & GINN, P. E. (2000) Hypercalcemia in a dog: a challenging case. *Journal of the American Animal Hospital Association* **36**, 20-25

ROSEN, L., ASH, L. R. & WALLACE, G. D. (1970) Life history of the canine lungworm *Angiostrongylus vasorum* (Baillet). *American Journal of Veterinary Research* **31**, 131-143

ROSOL, T. J., CHEW, D. J., NAGODE, L. A. & SCHENCK, P. (2000) Disorders of calcium: hypercalcemia and hypocalcemia. In: Fluid Therapy in Small Animal Practice. 2nd edn. Ed S. P. DiBartola. W. B. Saunders, Philadelphia. pp 108-162

SCHENCK, P. A., CHEW, D. J. & BROOKS, C. L. (1996) Fractionation of canine serum calcium using a micropartition system. *American Journal of Veterinary Research* **57**, 268-271

SHARMA, O. P. (2000) Hypercalcemia in granulomatous disorders: a clinical review. *Current Opinion in Pulmonary Medicine* **6**, 442-447

TROY, G. C., FORRESTER, D., COCKBURN, C., MORTON, L. D., STORTS, R., WILSON, E. & CRAIG, T. (1986) *Heterobilharzia americana* infection and hypercalcemia in a dog: a case report. *Journal of the American Animal Hospital Association* **23**, 35-40

Neosporosis in a young dog presenting with dermatitis and neuromuscular signs

A 16-week-old, male boxer dog developed multifocal nodular dermatitis followed by rapidly progressive and fatal neuromuscular disease. Protozoal tachyzoites were demonstrated by aspiration and biopsy of dermal lesions. Necropsy and histology revealed necrotising inflammation associated with intralesional protozoal organisms in various organs including the brain, heart, skeletal muscle and skin. Serology suggested active infection with *Neospora caninum*. Immunohistochemistry provided a definitive diagnosis. Dermatitis is a finding rarely associated with juvenile neosporosis. The possible role of immunosuppression is discussed.

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INTRODUCTION

Neospora caninum is an apicomplexan protozoan, now well recognised as a cause of neuromuscular disease in dogs and abortion in cattle (Dubey 2003). The dog has been identified as both an intermediate and definitive host to the parasite (McAllister and others 1998), with transmission being primarily transplacental or by ingestion of tissues containing *N caninum* cysts (Hemphill 1999).

Canine neosporosis has been documented worldwide, with estimates of prevalence varying widely, and an apparent association between canine and bovine infection has been reported (Wouda and others 1999, Dubey 2003). Surveys in the UK have estimated canine seropositivity at between 5·8 and 16·6 per cent, the majority of these infections being subclinical (Trees and others 1993, Lathe 1994).

Lesions in the neuromuscular system usually predominate. Progressive hindlimb paresis with muscle atrophy and rigid hindlimb hyperextension is a characteristic presentation in juvenile dogs (Barber and Trees 1996). However, tissue inflammation associated with tachyzoite replication can be widespread, particularly in peracute cases, and may include myocarditis, hepatitis, dermatitis, ocular lesions, pancreatitis and interstitial pneumonia (Dubey and others 1990b, Barber and others 1996, Barber and Trees 1996). Signs are likely to be more generalised in adult dogs (Barber and Trees 1996). Cutaneous involvement is infrequently reported but may be multifocal or occur as a localised lesion (Dubey and others 1995, Poli and others 1998).

The present report describes a fatal case of neosporosis in a puppy in which dermatitis manifested before signs of acute polymyositis and encephalitis.

CASE HISTORY

A 16-week-old, entire male, vaccinated, boxer dog presented to a private veterinary clinic with severe acute vomiting, pyrexia and bloody diarrhoea. *Salmonella* group D was cultured from its faeces. A 10-day course of 250 mg amoxycillin/clavulanate (Synulox; Pfizer), given orally twice daily, resulted in clinical recovery, although the frequency of urination was thought to be increased.

Three days after completion of the course of antibiotics, multiple erythematous non-pruritic dermal nodules, measuring 0·5 to 2 cm in diameter, developed in the ventrum and axillary regions. Physical examination was otherwise unremarkable. On suspicion of a hypersensitivity reaction, 0·25 mg/kg dexamethasone sodium phosphate (Colvasone; Norbrook) and 10 mg chlorpheniramine (Piriton; Glaxo-SmithKline) were administered subcutaneously.

Over the following two days the dog became dull, lethargic, polyuric, polydipsic and mildly febrile (39·4°C). Serum biochemistry showed a marked elevation in the level of alanine aminotransferase (ALT) (>1000 U/litre, reference range 8 to 75 U/litre) (VetTest; Idexx Laboratories). Urine was isosthenuric by refractometry (specific gravity 1·011). A urine dipstick (Multistix; Bayer) showed a pH of 8, trace protein and +++ blood. No erythrocytes were found on sediment examination. Abdominal ultrasound was unremarkable.

Treatment consisted of parenteral fluids (Aqupharm No. 11; Animalcare) at

Table 1. Biochemical findings in a boxer puppy with disseminated neosporosis

Analyte	Results	Reference values
Total protein	39·3	55-73 g/litre
Globulin	15·5	27-44 g/litre
Albumin	23·8	25-39 g/litre
Sodium	142	145-155 mmol/litre
Potassium	4·7	4-5·5 mmol/litre
Chloride	113	105-120 mmol/litre
Calcium	2·30	2·2-2·85 mmol/litre
Phosphorus	2·14	1-2·52 mmol/litre
Urea	6·2	3-10 mmol/litre
Creatinine	91	50-140 µmol/litre
Creatine kinase	135,009	80-500 U/litre
ALT	1099	22-220 U/litre
ALP	572	30-250 U/litre
Cholesterol	6·0	2·5-8 mmol/litre
Total bilirubin	4·0	2·5-12 µmol/litre
Amylase	968	350-1500 U/litre
Lipase	2321	55-430 U/litre
Bile acids	53·6	<10 µmol/litre

ALT Alanine aminotransferase, ALP Alkaline phosphatase

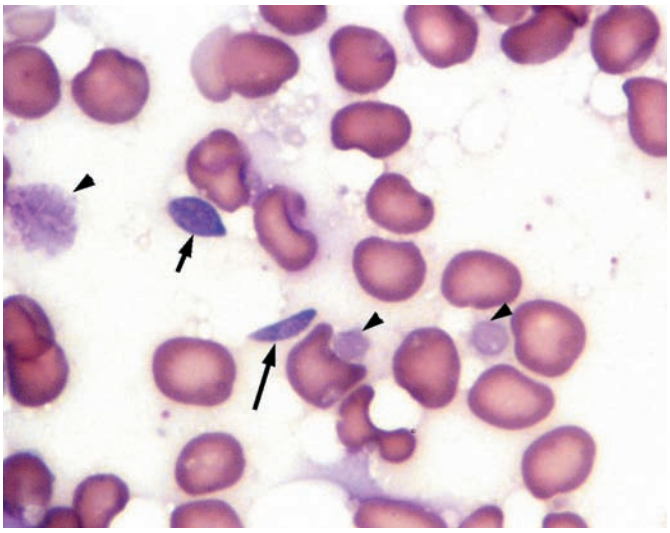

FIG 1. Needle aspirate of a skin lesion. Extracellular tachyzoites occurred singly (long arrow) or paired and undergoing division by endodyogeny (short arrow), with platelets (arrowheads) and erythrocytes. Diff-Quik. ×1000

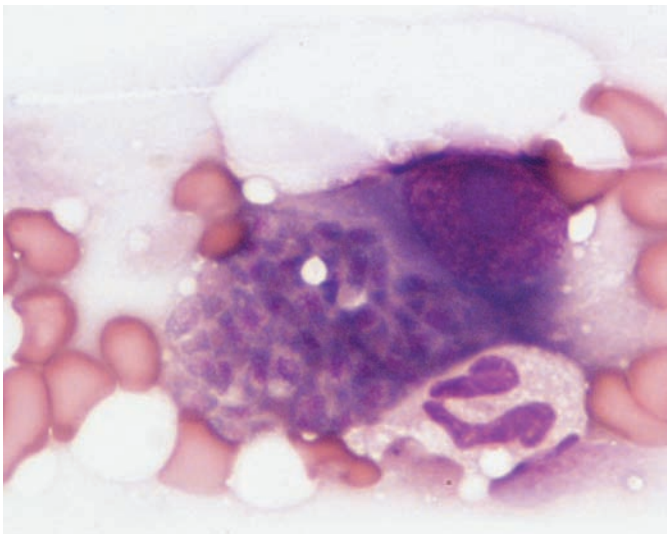

FIG 2. Needle aspirate of a skin lesion, showing a macrophage with cytoplasm distended by more than 30 tachyzoites. A neutrophil and erythrocytes are visible in the background. Diff-Quik. ×1000

maintenance rates, 5 mg/kg enrofloxacin (Baytril; Bayer) subcutaneously every 24 hours, and 2 mg/kg dexamethasone intravenously in divided doses over 24 hours. Mental dullness and weakness worsened, progressing over two days to paraparesis and lateral recumbency with dyspnoea and dysphagia. Further samples, consisting of aspirates and biopsy of dermal lesions, cerebrospinal fluid (CSF), blood and urine for culture, were sent to the Department of Pathology and Infectious Disease, at the Royal Veterinary College (RVC), London.

Haematology (Abbot Cell-dyn 3500CS) revealed a mild eosinophilia (2·14×10^9/litre, reference range 0 to 1·3×10^9/litre). Platelet clumping in the sample prevented an accurate count but numbers appeared adequate upon smear examination. Abnormal serum biochemical results included marked elevation of creatine kinase, moderate elevations of ALT, bile acids and lipase, a mild increase in alkaline phosphatase, hypoproteinaemia with moderate hypoglobulinaemia and mild hypoalbuminaemia, and mild hyponatraemia (Table 1).

Prothrombin time (PT), at 12·2 seconds, was prolonged 44 per cent above a canine control; activated partial thromboplastin time (APTT) was unremarkable at 17·5 seconds. Protein (0·11 g/litre, reference range <0·25 g/litre) and total nucleated cell counts (3×10^6/litre, reference range <10×10^6/litre) in CSF were unremarkable. A CSF nucleated cell differential count showed an increased proportion of neutrophils (45 per cent non-degenerate neutrophils, 30 per cent monocytoid cells and 25 per cent lymphocytes). However, this was attributed to moderate blood contamination in the sample.

Smears from aspirates of cutaneous nodules showed mixed inflammation (56 per cent neutrophils, 41 per cent eosinophils and 3 per cent macrophages) with occasional mast cells and reactive fibroblasts. Crescent-shaped organisms with an apical nucleus were scattered extracellularly throughout the smear, singly or dividing by endodyogeny (Fig 1). Organisms were also densely clustered within and expanding low numbers of macrophages (Fig 2). However, clinical deterioration was rapid and the pup became stuporous and died before a diagnosis of protozoal disease was made. Death occurred within six days of skin lesions appearing.

Histology of the skin biopsy subsequently revealed suppurative and eosinophilic nodular dermatitis and panniculitis, with patchy suppurative folliculitis and furunculosis. Protozoal organisms were found extracellularly and within histiocytic cells and fibroblasts (Fig 3). Urine and CSF cultures yielded no growth. Serology for *Toxoplasma gondii* was negative (Toxoreagent; Eiken Chemical Co.). An indirect fluorescent antibody test (IFAT) showed seropositivity to *N caninum* at 1:800. Serology for antinuclear antibody was negative.

A postmortem examination was performed at the RVC's Department of Pathology and Infectious Disease. Findings included widespread severe pale streaking

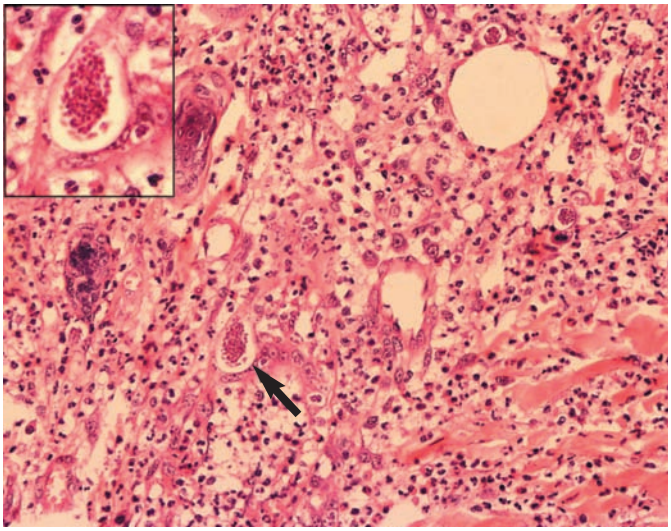

FIG 3. Section of dermis showing severe pyogranulomatous inflammation, with numerous admixed eosinophils. Protozoal organisms were observed within histiocytic cells (arrow and inset) and fibroblasts. Haematoxylin and eosin. ×200. Inset ×1000

of cardiac and skeletal muscles, suggesting diffuse myopathy. There were ulcerative skin lesions of limbs, the ventral abdomen, sternum, neck and head. The urinary bladder contained dark red urine. Multiple portions of brain, skeletal muscle including diaphragm, heart, skin, stomach, small intestine, urinary bladder, adrenals, kidneys, lungs, liver, spleen, pancreas, bone marrow and thyroid glands were fixed in 10 per cent buffered formalin, processed routinely, and sections were stained with haematoxylin and eosin for histological examination.

Histologically, the predominant lesions were multifocal necrotising myositis of skeletal and cardiac muscle, multifocal pyogranulomatous ulcerative necrotising dermatitis and multifocal nonsuppurative necrotising encephalitis. Multiple foci of necrosis were also found within the pancreas, adrenal cortices and intestinal smooth muscle. Peyer's patches were markedly depleted of lymphocytes. Epithelial cells of cortical renal tubules contained intracytoplasmic red granules suggestive of myoglobin. Similar material formed intratubular casts in the renal medullae. The liver and lungs were markedly congested. Hepatic Kupffer cells and splenic reticuloendothelial cells were prominent.

Protozoal tachyzoites were found within cardiac and skeletal myocytes, brain, pancreas and dermis. In the brain, encysted stages and tachyzoites were most abundant in the cerebrum. Immunohistochemical staining of skin, brain, skeletal and cardiac muscle was positive for *N caninum* and negative for *T gondii*.

DISCUSSION

Canine neosporosis characteristically presents as progressive hindlimb paresis and paralysis in juvenile dogs. The boxer is among those breeds in which neosporosis is most frequently reported (Cringoli and others 2002, Dubey 2003). Dermatitis is a rare presentation in canine juvenile neosporosis, and nine of 10 previously reported cases of dermal neosporosis involved adult dogs (Fritz and others 1997, Perl and others 1998, Poli and others 1998, La Perle and others 2001, Ordeix and others 2002).

Cutaneous lesions consist of multifocal, ulcerated and sometimes exudative nodules, measuring 0·5 to 5 cm in diameter with variable distribution, and are characterised by pyogranulomatous necrotising inflammation (Dubey and others 1988, 1995, La Perle and others 2001). Tachyzoites in skin lesions may be abundant, occurring extracellularly or within inflammatory or tissue cells including fibroblasts, macrophages and epithelial cells. Peripheral eosinophilia and dermal eosinophilic infiltrates, as occurred in the present case, are variable findings. Localised nodular dermatitis in the apparent absence of systemic illness has also been reported (Poli and others 1998).

Although skin lesions were the presenting complaint in this case, evidence of disseminated infection manifested rapidly. Intracellular tachyzoite replication and ensuing cell necrosis and inflammation were widespread. Extensive myonecrosis and cerebral and visceral invasion accounted for the majority of clinicopathological abnormalities, including the extreme creatine kinase elevation (Table 1). Myoglobinuria and presumed secondary tubular nephropathy were considered a probable cause of polyuria, polydipsia and isosthenuria. Hypoproteinaemia in puppies may reflect immaturity of production (Stockham and Scott 2002). However, the level in this case was lower than expected and primarily reflected hypoglobulinaemia, the cause of which may have been clarified by measuring globulin fractions.

A role for immunosuppression in the development of cutaneous lesions in neosporosis has previously been proposed (Dubey and others 1995, La Perle and others 2001, Ordeix and others 2002). Simultaneous skin infection with *N caninum* and *Leishmania infantum* has been reported in Italy in a nine-month-old dog on steroid therapy (Tarantino and others 2001). Other reports involve aged dogs, those with concurrent disease, or dogs on chronic immunosuppressive therapy for immune-mediated or neoplastic disease (Dubey and others 1988, 1995).

Cell-mediated immunity involving interferon-gamma, produced by CD4+ T helper type 1 lymphocytes and macrophages, significantly inhibits intracellular tachyzoite replication (Innes and others 2000). Activation of latent tissue cysts and tachyzoite replication may occur if host immune responses are altered, as occurs during pregnancy, immunosuppressive therapy or concurrent disease (Dubey and Lindsay 1990, Dubey and others 1995, Buxton and others 2002). Immunity in the present case may have been compromised by a number of factors including immaturity, previous infection with *Salmonella* and corticosteroid administration. Glucocorticoids may impair cell-mediated immunity via down-regulation of macrophage function and T lymphocyte suppression (Ferguson and Hoenig 2001). It can only be speculated whether the episode of *Salmonella*-induced gastroenteritis triggered activation of latent protozoal disease or resulted from pre-existing immunocompromise in this young boxer dog.

Aspiration and biopsy of skin lesions may demonstrate protozoal organisms in dermal neosporosis. In addition to *N caninum*, protozoal species reported to cause dermatitis in dogs are *Leishmania* species, *Sarcocystis canis* and *Caryospora* species. Serology, organism morphology and immunohistochemistry aid differentiation of these parasites (Dubey and others 1990a, Dubey and Speer 1991). Although not reported to cause dermal lesions in dogs, tachyzoites of *T gondii* are indistinguishable from those of *N caninum* by light microscopy (Dubey and others 1995). Toxoplasmosis may cause generalised disease in dogs resembling neosporosis and dual infection can occur (Dubey and others 1995). Recently an unidentified protozoal parasite was associated with ulcerative nodular dermatitis in an adult dog in Brazil (Dubey and others 2003). Although staining positively for *T gondii* polyclonal antibody, organisms divided by schizogony and were ultrastructurally distinct from *T gondii*, suggesting that a new entity should be considered in the differential diagnoses of protozoal dermatitis in dogs.

Serology by IFAT demonstrates very little cross-reactivity between *N caninum* and other coccidian parasites (Bjorkman and Uggla 1999). Clinical neosporosis in most cases produces IFAT titres greater than or equal to 1:800 (Barber and Trees 1996). Definitive diagnosis requires immunohistochemistry or electron microscopy of tissue sections.

An interesting finding in this case was the prolongation of PT. Coagulation times are infrequently reported in neosporosis. Prolonged PT due to acquired factor VII deficiency can occur in cattle infected with the closely related protozoa of *Sarcocystis* species (Dubey 1993). However, the incidence of coagulopathy in canine neosporosis is unknown. Prolonged PT with normal APTT suggests a deficiency of factor VII, which, due to the short half-life of this factor, may be an early finding with loss of hepatic factor production or decreased intestinal uptake of vitamin K. In this case, coagulopathy was not clinically apparent and a detailed assessment of coagulation was not conducted.

Successful treatment of neosporosis with clindamycin, sulphadiazine and pyrimethamine, alone or in combination, has been reported (Dubey and others 1995, Barber and Trees 1996, Poli and others 1998, Ordeix and others 2002). However, the prognosis in acute generalised disease is guarded. Localised disease and early instigation of therapy are associated with a better outcome (Dubey and others 1995, Barber and Trees 1996).

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References

Barber, J. S., Payne-Johnson, C. E. & Trees, A. J. (1996) Distribution of *Neospora caninum* within the central nervous system and other tissues of six dogs with clinical neosporosis. *Journal of Small Animal Practice* **37**, 568-574

Barber, J. S. & Trees, A. J. (1996) Clinical aspects of 27 cases of neosporosis in dogs. *Veterinary Record* **139**, 439-443

Bjorkman, C. & Uggla, A. (1999) Serological diagnosis of *Neospora caninum* infection. *International Journal for Parasitology* **29**, 1497-1507

Buxton, D., McAllister, M. M. & Dubey, J. P. (2002) The comparative pathogenesis of neosporosis. *Trends in Parasitology* **18**, 546-552

Cringoli, G., Rinaldi, L., Capuano, F., Baldi, L., Veneziano, V. & Capelli, G. (2002) Serological survey of *Neospora caninum* and *Leishmania infantum* co-infection in dogs. *Veterinary Parasitology* **106**, 307-313

Dubey, J. P. (1993) *Toxoplasma*, *Neospora*, *Sarcocystis*, and other tissue cyst-forming coccidia of humans and animals. In: Parasitic Protozoa, Vol 6. 2nd edn. Ed J. P. Kreier. Academic Press, San Diego. pp 92-94

Dubey, J. P. (2003) Review of *Neospora caninum* and neosporosis in animals. *Korean Journal of Parasitology* **41**, 1-16

Dubey, J. P., Black, S. S., Sangster, L. T., Lindsay, D. S., Sundermann, C. A. & Topper, M. J. (1990a) *Caryospora*-associated dermatitis in dogs. *Journal of Parasitology* **76**, 552-556

Dubey, J. P., Carpenter, J. L., Speer, C. A., Topper, M. J. & Uggla, A. (1988) Newly recognized fatal protozoan disease of dogs. *Journal of the American Veterinary Medical Association* **192**, 1269-1285

Dubey, J. P., Koestner, A. & Piper, R. C. (1990b) Repeated transplacental transmission of *Neospora caninum* in dogs. *Journal of the American Veterinary Medical Association* **197**, 857-860

Dubey, J. P. & Lindsay, D. S. (1990) Neosporosis in dogs. *Veterinary Parasitology* **36**, 147-151

Dubey, J. P., Metzger, F. L., Jr, Hattel, A. L., Lindsay, D. S. & Fritz, D. L. (1995) Canine cutaneous neosporosis: clinical improvement with clindamycin. *Veterinary Dermatology* **6**, 37-43

Dubey, J. P., Pimenta, A. L., Abboud, L. C. S., Ravasani, R. R. & Mense, M. (2003) Dermatitis in a dog associated with an unidentified *Toxoplasma gondii*-like parasite. *Veterinary Parasitology* **116**, 51-59

Dubey, J. P. & Speer, C. A. (1991) *Sarcocystis canis* n. sp. (Apicomplexa: Sarcocystidae), the etiologic agent of generalized coccidiosis in dogs. *Journal of Parasitology* **77**, 522-527

Ferguson, D. C. & Hoenig, M. (2001) Glucocorticoids, mineralocorticoids, and steroid synthesis inhibitors. In: Veterinary Pharmacology and Therapeutics. 8th edn. Ed H. R. Adams. Iowa State University Press, Iowa. p 653

Fritz, D., George, C., Dubey, J. P., Trees, A. J., Barber, J. S., Hopfner, C. L., Mehaut, S., Le Net, J. L. & Longeart, L. (1997) *Neospora caninum*: associated nodular dermatitis in a middle-aged dog. *Canine Practice* **22**, 21-24

Hemphill, A. (1999) The host-parasite relationship in neosporosis. *Advances in Parasitology* **43**, 47-103

Innes, E. A., Buxton, D., Maley, S., Wright, S., Marks, J., Esteban, I., Rae, A., Schock, A. & Wastling, J. (2000) Neosporosis: aspects of epidemiology and host immune response. *Annals of the New York Academy of Sciences* **916**, 93-101

La Perle, K. M. D., Del Piero, F., Carr, R. F., Harris, C. & Stromberg, P. C. (2001) Cutaneous neosporosis in two adult dogs on chronic immunosuppressive therapy. *Journal of Veterinary Diagnostic Investigation* **13**, 252-255

Lathe, C. L. (1994) *Neospora caninum* in British dogs. *Veterinary Record* **134**, 532

McAllister, M. M., Dubey, J. P., Lindsay, D. S., Jolley, W. R., Wills, R. A. & McGuire, A. M. (1998) Dogs are definitive hosts of *Neospora caninum*. *International Journal for Parasitology* **28**, 1473-1478

Ordeix, L., Lloret, A., Fondevila, D., Dubey, J. P., Ferrer, L. & Fondati, A. (2002) Cutaneous neosporosis during treatment of pemphigus foliaceus in a dog. *Journal of the American Animal Hospital Association* **38**, 415-419

Perl, S., Harrus, S., Satuchne, C., Yakobson, B. & Haines, D. (1998) Cutaneous neosporosis in a dog in Israel. *Veterinary Parasitology* **79**, 257-261

Poli, A., Mancianti, F., Carli, M. A., Stroscio, M. C. & Kramer, L. (1998) *Neospora caninum* infection in a Bernese cattle dog from Italy. *Veterinary Parasitology* **78**, 79-85

Stockham, S. L. & Scott, M. A. (2002) Proteins. In: Fundamentals of Veterinary Clinical Pathology. Iowa State University Press, Iowa. pp 251-276

Tarantino, C., Rossi, G., Kramer, L. H., Perruci, S., Cringoli, G. & Macchioni, G. (2001) *Leishmania infantum* and *Neospora caninum* simultaneous skin infection in a young dog in Italy. *Veterinary Parasitology* **102**, 77-83

Trees, A. J., Guy, F., Tennant, B. J., Balfour, A. H. & Dubey, J. P. (1993) Prevalence of antibodies to *Neospora caninum* in a population of urban dogs in England. *Veterinary Record* **132**, 125-126

Wouda, W., Dijkstra, T., Kramer, A. M. H., van Maanen, C. & Brinkhof, J. M. A. (1999) Seroepidemiological evidence for a relationship between *Neospora caninum* infections in dogs and cattle. *International Journal for Parasitology* **29**, 1677-1682

Ethmoidal encephalocoele associated with seizures in a puppy

A six-month-old puppy was presented for investigation of a seizure disorder. Neurological examination indicated persistent cerebral dysfunction in the absence of any identifiable metabolic disorder and magnetic resonance imaging revealed extension of the rostral lobes of the cerebrum into the nasal cavity. Despite symptomatic treatment, the puppy continued to exhibit seizures and appeared distressed and so was euthanased. Postmortem examination confirmed the abnormal anatomy of the rostral part of the brain and absence of a cribriform plate. There was extensive grey and white matter degeneration plus intraparenchymal haemorrhage in the abnormal brain tissue. The findings are consistent with a diagnosis of ethmoidal encephalocoele – a condition that has not previously been reported in the dog.

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INTRODUCTION

There are numerous causes of seizures in dogs but when dealing with juvenile animals the possibility of a congenital lesion should be given particular consideration (Oliver and others 1997). Extracranial lesions, such as portosystemic shunts, are usually easily identified through blood analysis, whereas intracranial lesions generally require sophisticated imaging techniques to achieve diagnosis.

The central nervous system (CNS) has a uniquely intricate architecture upon which its function is critically dependent. Therefore, abnormalities that occur during development of the brain are particularly liable to cause deficits and derangements in function that commonly become manifest as seizures. Several types of brain malformation have been identified in dogs, such as lissencephaly and polymicrogyria (Zaki 1976, van Winkle and others 1994, Saito and others 2002), although most common by far is hydrocephalus. Such lesions can often be identified using computed tomography (CT) or magnetic resonance imaging (MRI) (Harrington and others 1996, Saito and others 2002).

This case report describes an unusual brain malformation in a puppy, which was associated with seizures and intractable cognitive deficits.

CASE HISTORY

A six-month-old, male crossbreed dog was referred to the Queen's Veterinary School Hospital (QVSH) for treatment of a seizure disorder. The owner had owned the puppy since it was eight weeks old, during which time it had been generally in good health, although it had had several seizures from which it had recovered uneventfully. One week before presentation to the clinic, the puppy had a further series of seizures, culminating in a prolonged period of disorientation and circling.

On initial presentation at the QVSH, the puppy walked compulsively in tight circles to the right. He appeared demented and barked continuously. Postural reactions were depressed in all four limbs and there was hypermetria of the thoracic limbs, especially the left. The menace response was absent in the left eye and equivocally depressed in the right; there were no other signs of cranial nerve dysfunction. Myotatic and flexor reflexes were intact in all of the limbs.

On routine biochemical analysis, blood urea was below the normal range but no other abnormalities were found; a bile acid stimulation test was normal. After hospitalisation the puppy's apparently distressed state was partially alleviated by frequent administration of parenteral diazepam, but the response diminished over the following days.

An MRI scan revealed grossly abnormal conformation of the brain in the region of the olfactory bulb and prefrontal cerebral cortex. On horizontal slices, tissue isointense and contiguous with normal brain extended rostrally far beyond the normal limits of the cranial vault to lie within the nasal cavity (Fig 1a and b). Although nearly symmetrical, the abnormality was most marked on the right side, where the

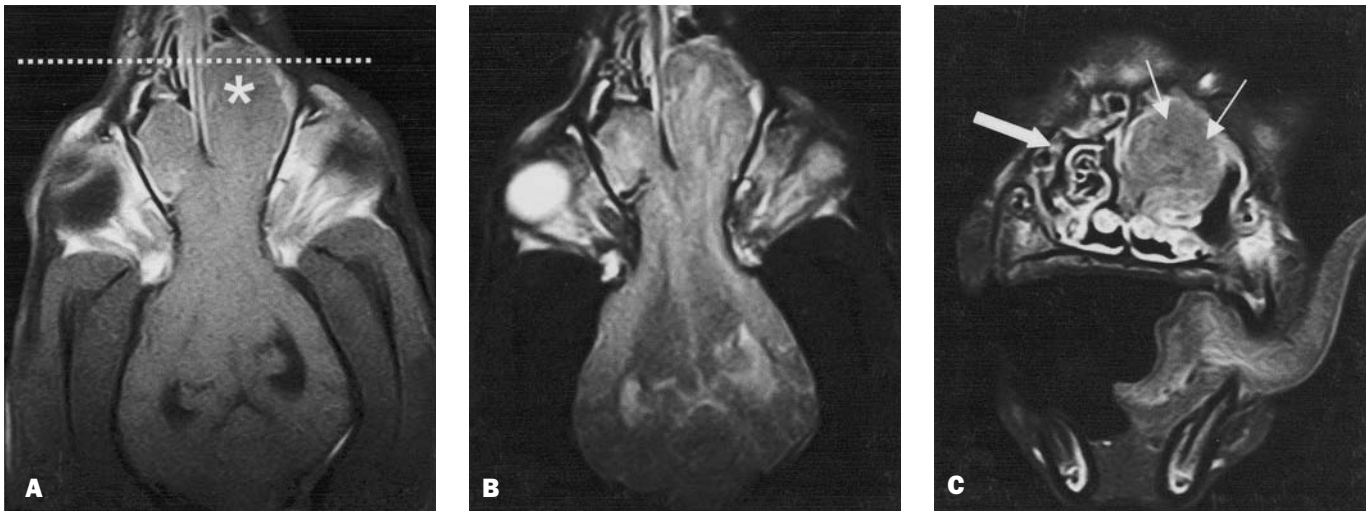

FIG 1. Magnetic resonance imaging (MRI) scans of the puppy's brain. (A) Horizontal T1-weighted scan. Asterisk shows location of normal-appearing brain tissue extending into an abnormal position rostral to the eye. (B) Horizontal T2-weighted scan of the same region, illustrating variable intensity of herniated brain. (C) Transverse T1-weighted MRI scan through the level indicated in (A) by the dashed line. Note the turbinates in left nasal cavity (large arrow), and hypointense (darker) regions suggestive of recent intraparenchymal haemorrhage (small arrows)

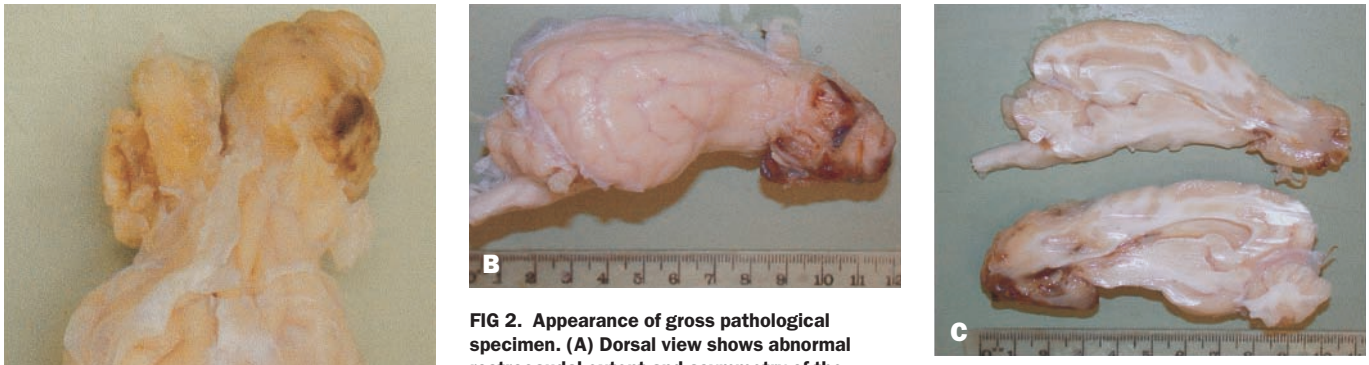

FIG 2. Appearance of gross pathological specimen. (A) Dorsal view shows abnormal rostrocaudal extent and asymmetry of the rostral part of the brain. Intimate attachment between the nasal mucosa and herniated brain is apparent in (B), a view of the right side of the brain, and in (C), which shows parasagittal slices of both cerebral hemispheres. Haemorrhage is also grossly apparent on the cut surfaces

rostral-most part of the brain lay approximately 1 cm rostral to the medial canthus. On transverse slices, normal-appearing brain tissue lay adjacent to nasal turbinates within the rostral part of the nasal cavity (Fig 1c). There was no discernible cribriform plate. On T2-weighted transverse scans, the frontal sinuses were filled with material of a hyperintense signal, consistent with accumulated fluid. Intravenous administration of 0·1 mmol/kg gadobenate dimeglumine (Multihance; Braco) did not reveal any evidence of a breakdown of the blood/brain barrier within the herniated portion of the brain. No other abnormalities were detected in the remaining portion of the brain and there was no evidence of either subtentorial or foramen magnum herniation.

During the following two days, attempts were made to control the puppy's symptoms using diazepam, acepromazine, pentobarbitone and phenobarbitone. However, while temporarily controlling the signs, upon recovery from sedation the puppy resumed the distressed behaviour and therefore was euthanased. Immediately after euthanasia, the puppy was perfused through the heart with formalin and the brain removed for histopathological examination.

Histopathological examination

The gross appearance of the brain was similar to that predicted from the MRI scan. The two frontal/olfactory lobes were greatly elongated and each ended in a bulb-like structure that was inseparable from the nasal mucosa; there was no cribriform plate. The brain was cut mid-sagittally; the right side frontal/olfactory region was sectioned sagittally and the left sectioned transversely (Fig 2). The corpus callosum was normal in location but somewhat distorted so that it was displaced rostrally towards the defect in the skull. The gross structure of the remaining portion of the brain was unremarkable.

Haemorrhage was evident to the naked eye in parasagittal sections and appeared to

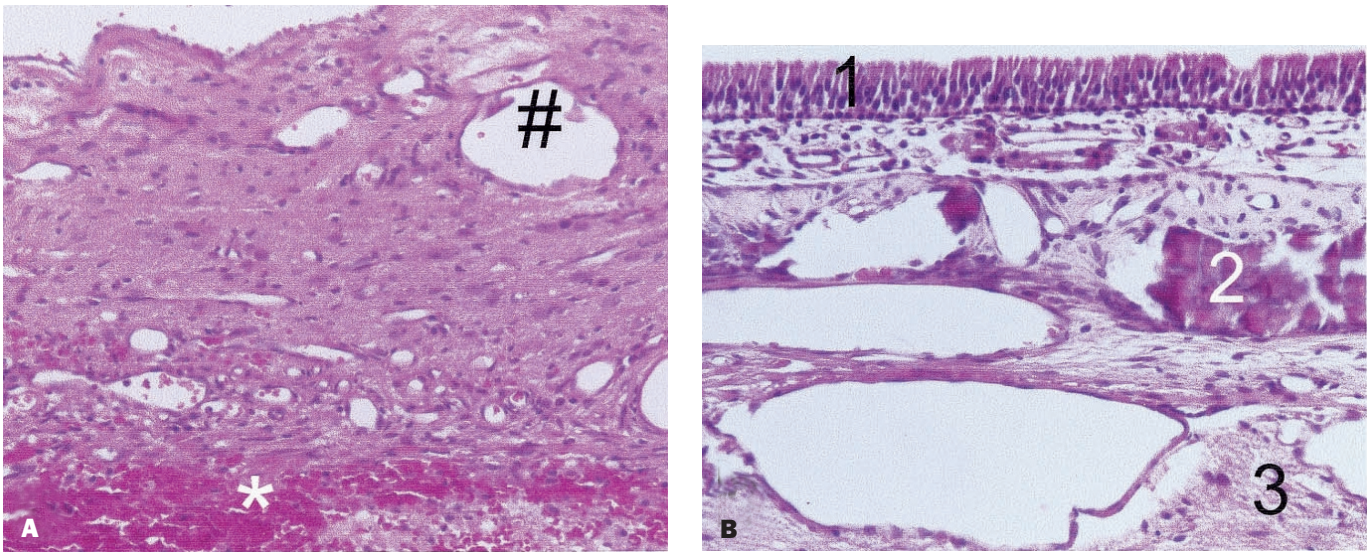

FIG 3. Microscopic appearance of the periphery of the herniated brain. (A) Beneath a thin columnar epithelium are numerous large blood vessels (#) contained within a mucosal structure. Haemorrhage is visible in the deeper central nervous system (CNS) tissue (*). Haematoxylin & eosin (H&E). ×50. (B) Columnar epithelium (1) typical of nasal mucosa; there is some fibrocartilaginous material in deeper layers (2) suggestive of a rudimentary cribriform plate. Normal-appearing CNS tissue (3) lies immediately beneath the mucosal layer. H&E. ×150

extend from the rostral tip of the ventricle into the subarachnoid space. There were also scattered, smaller regions of subdural haemorrhage. On microscopic examination, the surface of the herniated brain was covered by tissue that resembled nasal mucosa and contained many large, thin-walled blood vessels beneath a layer of columnar epithelium (Fig 3). Several regions of white matter showed extensive Wallerian degeneration, most notably where the dorsoventral dimensions of the protruding rostral lobes were maximally reduced. There were several discrete areas of malacia and evidence of previous haemorrhage and hypercellularity.

DISCUSSION

The malformation described in this puppy fulfils the criteria to be described as a congenital encephalocoele. This malformation is recognised as one category of neural tube defects in which part of the brain herniates through an abnormal opening in the cranium (David and Proudman 1989, Harding and Copp 1997).

Encephalocoele is well recognised in human infants, occurring at a frequency of approximately nine in 100,000 live births in the UK (Kadir and others 1999), although the rate of occurrence is much higher in Asia (Suwanwela and Suwanwela 1972, Hoving 2000). There are several subtypes recognised in humans, categorised according to which part of the cranium is affected (Harding and Copp 1997). Those affecting the occipital region are most common, while those of the frontal and ethmoidal region, such as occurred in the puppy in the present case, are rarest (Ziter and Bramwit 1970, Boonvisut and others 1998, Hoving 2000). Encephalocoele has previously been recognised in pigs as an inherited condition (Vogt and others 1986) and in cats as a complication of antenatal treatment with griseofulvin (Scott and others 1975). The one previous case report on encephalocoele in a dog (Parker and Cusick 1974) described somewhat different clinical signs: a two-day-old puppy was presented comatose, with a red mass overlying the parietal region and was immediately euthanased. Furthermore, since that case report predates veterinary access to advanced imaging, the MRI characteristics of this disorder have not previously been described in animals.

Encephalocoele is thought to develop because of a failure of neurulation, during the first third of gestation, in which there is incomplete separation between the neurectoderm and the overlying mesoderm (Hoving 2000). The precise means by which this occurs are unknown, but genetic factors are certainly implicated in view of the varying incidence in different parts of the world. Environmental factors are also involved since there has been a notable reduction in incidence of encephalocoele (and other neural tube deficits) in both the USA and UK since the addition of folic acid to food (Kadir and others 1999). Experimentally, excessive vitamin A can also cause encephalocoele (Theodosis and Fraser 1978).

For many developmental abnormalities of the brain, it is clear why seizures should develop, for instance because of pressure changes in the ventricles (hydrocephalus) or abnormal 'wiring' patterns (lissencephaly). In the puppy in the present case, it is difficult at first sight to understand why seizures should have occurred, especially since the puppy appeared normal when younger. Pathological examination of the herniated brain tissue provides an answer, revealing gross haemorrhage, malacia, degeneration of white matter and inflammatory cell infiltrate that could all contribute to abnormal excitability of cortical neurons. It could also be postulated that the very vascular mucosa was more prone to trauma, especially in its site within the nasal cavity, and the lack of a distinct barrier between the nasal mucosa and the brain could have allowed easy access to microbes.

In human infants encephalocoeles are treated by excision of the herniated brain

and closure of the defect in the skull (Boonvisut and others 2001, Hunt and Hobar 2003). Future cases of encephalocoele in dogs may prove amenable to such treatment, but owing to the intractable nature of the clinical signs in the current case it appeared inhumane to further prolong the puppy's life.

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References

Boonvisut, S., Ladpli, S., Sujatanond, M., Tandhavadhana, C., Tisavipat, N., Luxsuwong, M., Nunta-aree, S., Srimaharaja, S., Panitphong, T., Dulayajinda, D. & Areewattana, S. (1998) Morphologic study of 120 skull base defects in frontoethmoidal encephalomeningoceles. *Plastic and Reconstructive Surgery* **101**, 1784-1795

Boonvisut, S., Ladpli, S., Sujatanond, M., Tisavipat, N., Luxsuwong, M., Nunta-aree, S., Boonampol, D., Srimaharaja, S., Panitphong, T., Dulayajinda, D. & Areewattana, S. (2001) A new technique for the repair and reconstruction of frontoethmoidal encephalomeningoceles by medial orbital composite-unit translocation. *British Journal of Plastic Surgery* **54**, 93-101

David, D. J. & Proudman, T. W. (1989) Cephaloceles: classification, pathology, and management. *World Journal of Surgery* **13**, 349-357

Harding, B. & Copp, A. J. (1997) Malformations. In: Greenfield's Neuropathology. Eds D. I. Graham and P. L. Lantos. Arnold, London. pp 397-533

Harrington, M. L., Bagley, R. S. & Moore, M. P. (1996) Hydrocephalus. *Veterinary Clinics of North America: Small Animal Practice* **26**, 843-856

Hoving, E. W. (2000) Nasal encephaloceles. *Child's Nervous System* **16**, 702-706

Hunt, J. A. & Hobar, P. C. (2003) Common craniofacial anomalies: facial clefts and encephaloceles. *Plastic and Reconstructive Surgery* **112**, 606-615

Kadir, R. A., Sabin, C., Whitlow, B., Brockbank, E. & Economides, D. (1999) Neural tube defects and periconceptional folic acid in England and Wales: retrospective study. *British Medical Journal* **319**, 92-93

Oliver, J. E., Lorenz, M. D. & Kornegay, J. N. (1997) Seizures and narcolepsy. In: Handbook of Veterinary Neurology. 3rd edn. Eds J. E. Oliver, M. D. Lorenz and J. N. Kornegay. W. B. Saunders, Philadelphia. pp 313-331

Parker, A. J. & Cusick, P. K. (1974) Meningoencephalocele in a dog (a case history). *Veterinary Medicine & Small Animal Clinician* **69**, 206-207

Saito, M., Sharp, N. J., Kortz, G. D., de Lahunta, A., Leventer, R. J., Tokuriki, M. & Thrall, D. E. (2002) Magnetic resonance imaging features of lissencephaly in 2 Lhasa Apsos. *Veterinary Radiology & Ultrasound* **43**, 331-337

Scott, F. W., LaHunta, A., Schultz, R. D., Bistner, S. I. & Riis, R. C. (1975) Teratogenesis in cats associated with griseofulvin therapy. *Teratology* **11**, 79-86

Suwanwela, C. & Suwanwela, N. (1972) A morphological classification of sincipital encephalomeningoceles. *Journal of Neurosurgery* **36**, 201-211

Theodosis, D. T. & Fraser, F. C. (1978) Early changes in the mouse neuroepithelium preceding exencephaly induced by hypervitaminosis A. *Teratology* **18**, 219-232

van Winkle, T., Fyfe, J., Dayrell-Hart, B., Aguirre, G., Acland, G. & Patterson, D. (1994) Blindness due to polymicrogyria and asymmetrical dilatation of the lateral ventricles in standard poodles. *Progress in Veterinary Neurology* **5**, 66-71

Vogt, D. W., Ellersieck, M. R., Deutsch, W. E., Akremi, B. & Islam, M. N. (1986) Congenital meningocele-encephalocele in an experimental swine herd. *American Journal of Veterinary Research* **47**, 188-191

Zaki, F. A. (1976) Lissencephaly in Lhasa Apso dogs. *Journal of the American Veterinary Medical Association* **169**, 1165-1168

Ziter, M. H. & Bramwit, D. N. (1970) Nasal encephaloceles and gliomas. *British Journal of Radiology* **43**, 136-138

Intracranial haemorrhage associated with *Angiostrongylus vasorum* infection in three dogs

This report describes three dogs with intracranial haemorrhage secondary to severe coagulation defects associated with *Angiostrongylus vasorum* infection. The initial case was diagnosed at necropsy, with two subsequent cases diagnosed antemortem and successfully treated. The dogs ranged in age from 14 months to four years and were presented for evaluation of a severe, subacute onset of suspected cerebral disease. Magnetic resonance imaging performed on all three dogs was suggestive of multiple areas of intraparenchymal brain haemorrhage. Coagulation assays showed a consumptive coagulopathy resembling chronic disseminated intravascular coagulation. Postmortem examination of the initial case confirmed the presence of multiple intracranial and extracranial haemorrhages. An unexpected finding was that of a marked multifocal nematode infection of the lungs with an associated vasculopathy. The parasites were confirmed to be *A vasorum.* In the two other dogs, faecal examination by Baermann technique confirmed *A vasorum* infection. Both dogs were treated with fenbendazole and one was additionally given a plasma transfusion. Repeated coagulation assays were normal within one week. Neurological examinations were normal for both dogs within six weeks. This case series indicates that *A vasorum* infection should be considered as a possible aetiology of intracranial haemorrhage in dogs.

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INTRODUCTION

Angiostrongylus vasorum, the French heartworm, is a metastrongyloid lungworm, which parasitises the right side of the heart and pulmonary arteries of domestic dogs and related wild carnivores. Infection requires the ingestion of slugs or snails, which act as intermediate hosts (Simpson and Neal 1982, Murdock 1984, Bolt and others 1994). After ingestion, the third-stage (L3) larvae migrate via the mesenteric lymph nodes and the liver, where they undergo further moults before passing into the right ventricle and pulmonary arteries. The adults are ovoviviparous, their eggs being transported within the pulmonary circulation to alveolar capillaries where they hatch as first-stage (L1) larvae. L1 larvae emerge into the alveoli and migrate to the trachea from where they are carried by upper airway mucociliary escalator to the trachea and expectorated or passed through the alimentary tract and excreted in faeces (Bolt and others 1994).

Clinical signs seen in association with *A vasorum* infection are variable and asymptomatic animals have been identified in naturally infected (Simpson and Neal 1982, Martin and others 1993) and experimentally infected cases (Prestwood and others 1981). Coughing, dyspnoea and exercise intolerance are common, but a variety of other signs, which may overshadow the signs of pulmonary disease, have been recorded and include stunted growth, weight loss, vomiting, lameness and subcutaneous swelling (Dodd 1973, Jones and others 1980, Simpson and Neal 1982, Patteson and others 1987, Cobb and Fisher 1990, Martin and others 1993, Bolt and others 1994). Additionally, sudden death attributable to acute heart failure has been reported (King and others 1994). Neurological disease including central vestibular signs, forelimb or hindlimb paralysis and acute lumbar pain has been described and attributed to embolism, although not confirmed during postmortem examination despite the presence of adult *A vasorum* in the systemic circulation (Perry and others 1991, Martin and others 1993, Patteson and others 1993).

Pathology and clinical reports have indicated that there may be a possible relationship between *A vasorum* infection and host coagulation abnormalities (Schelling and others 1986, Ramsey and others 1996, Gould and others 1999). Furthermore, a consumptive coagulopathy, with thrombocytopenia, resembling chronic disseminated intravascular coagulation has been demonstrated in a case of naturally occurring *A vasorum* infection (Ramsey and others 1996).

This report describes three dogs with intracranial haemorrhage secondary to severe coagulation defects associated with *A vasorum* infection.

CASE HISTORIES

All cases were referred to the Neurology Unit at the Animal Health Trust, Newmarket.

Case 1

A 14-month-old, female entire English springer spaniel was referred as an emergency for investigation of an acute onset of behavioural abnormalities and generalised weakness. No trauma was noted prior to clinical signs. The owners reported no other prior health problems or known exposure to rodenticides, and vaccinations were up to date.

The dog's behaviour had suddenly changed 10 days before referral at which time the owners had identified a fluctuant mass over the left ischium. The mass was seen to progressively increase in size over the next two days when the dog exhibited a gait abnormality in the right pelvic limb. A fine-needle aspirate of the mass was performed by the referring veterinarian, and identified a predominance of red blood cells without evidence of erythrocytophagia or malignancy. Seven days before referral, the dog experienced a sudden collapse, and although there was no loss of consciousness, a generalised transient tonic episode was observed. Over the following two days, the dog's vision became reduced, a sign which was accompanied by the presence of bilateral scleral haemorrhages. The dog became rapidly more depressed, anorexic and generally weak over the next few days until the time of referral.

On presentation, the dog exhibited a severely depressed mental status and non-ambulatory tetraparesis. Scleral haemorrhages were identified on both eyes with marked swelling of both optic discs. A 5 cm diameter painless subcutaneous mass, which was reasonably mobile on palpation, was located over the left ischium. There was no evidence of echymoses or petechiation on any of the mucosal membranes. Postural reactions were abnormal on all four limbs but were most affected on the left side. Spinal reflex examination was normal. Cranial nerve examination revealed vertical positional nystagmus, bilateral mydriasis and bilateral menace response deficits with absent pupillary light reflexes. A diffuse or multifocal forebrain and brainstem anatomical diagnosis was considered on the basis of the neurological findings. Inflammatory or infectious central nervous system disease, primary or metastatic neoplasia and cerebrovascular disease were considered as the more likely differentials for this neurolocalisation and clinical history.

Haematology and serum chemistry analyses were within normal limits, but a platelet count and coagulation profile were not performed prior to anaesthesia for intracranial imaging. The dog was anaesthetised using intravenous propofol (Rapinovet; Schering-Plough) induction to effect and maintained on isoflurane gas (IsoFlo; Abbot Animal Health). Magnetic resonance imaging (MRI) of the brain was performed with a 1·5 Tesla superconducting magnet (Wipro GE Healthcare 1.5T signa MRI) using a human extremity coil. A standard brain protocol was performed including the following pulse sequences: pre- and post-contrast (following intravenous administration of 0·1 mmol/kg gadopentetate dimeglumine [Omniscan; Nycomed]), T1-weighted, T2-weighted, T2*-gradient echo and proton density images.

A 2 cm solitary intra-axial mass was detected within the ventrolateral aspect of the left frontal lobe with extensive white matter oedema. The mass was complex in structure with a thin capsule of hyperintense tissue on T1- and T2-weighted images. The periphery of the mass was isointense to grey matter on T2-weighted images and slightly hyperintense on T1-weighted images. The centre of the mass was irregular with predominantly low to mid signal intensity on T2-weighted scans with interspersed areas of hyperintensity. On T1-weighted scans, the centre of the mass was isointense to grey matter with focal mild hyperintensities. The mass was predominantly hypointense on gradient echo images with the exception of a small ovoid area ventrolateral to the right of the mass which was hyperintense. Post-contrast images showed mildly increased meningeal enhancement but no enhancement of the mass. A diagnosis of subacute intracerebral haemorrhage was made based on these MRI findings.

A clotting profile revealed a markedly prolonged prothrombin time (PT) of 48 seconds (reference range seven to 10 seconds), activated partial thromboplastin time (APTT) of 51·3 seconds (reference range 11 to 18 seconds) and thrombin clotting time (TCT) of 27 seconds (reference range four to 6·5 seconds). Differentials included disorders of the common clotting pathway such as congenital coagulation disorders (Factor I and X deficiency), vitamin K antagonism, hepatobiliary disease and disseminated intravascular coagulation due to inflammatory, infectious, neoplastic or parasitic disease. No additional diagnostic tests were performed as the owners elected for euthanasia due to the rapid progression of the clinical signs. A postmortem examination confirmed an organising focal intracerebral haemorrhage. A large haematoma was present in the subcutis of the dorsal lumbosacral area. There was also evidence of prior haemorrhage and blood breakdown affecting lymph nodes and the urinary bladder. An unexpected finding was that of a myocarditis affecting the right auricle and marked multifocal nematode infection of the lungs with an associated pulmonary vasculopathy (Fig 1). The parasites were confirmed to be *A vasorum*.

Case 2

A four-year-old, male neutered cocker spaniel was referred for investigation of a 13-day history of acute-onset left-sided weakness, loss of vision in the left eye and abnormal mental status. Seven weeks prior

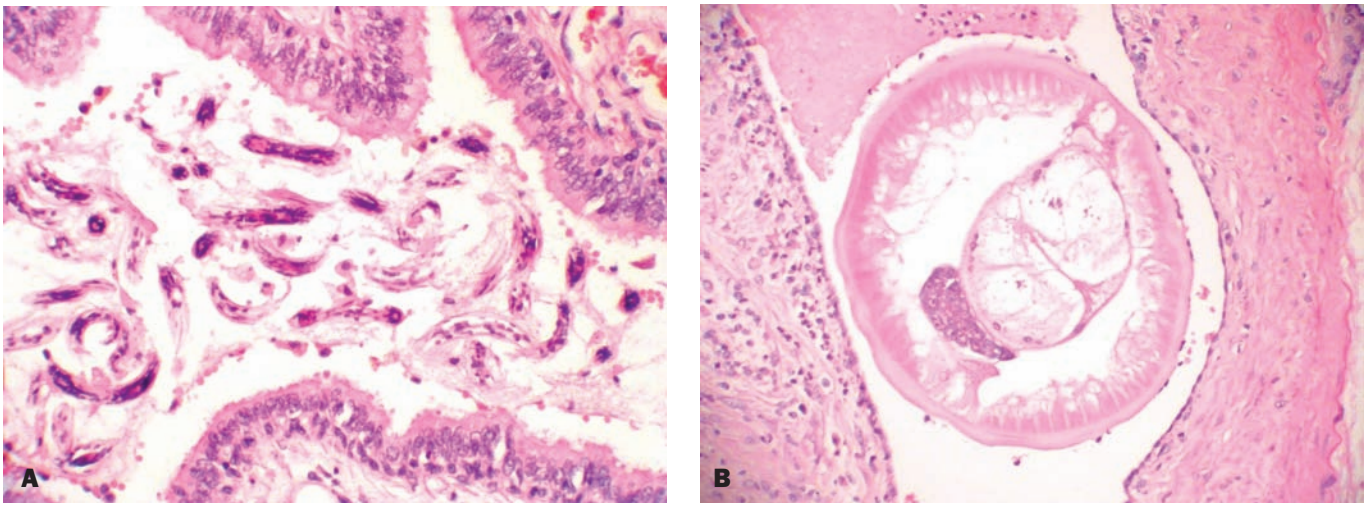

FIG 1. Histological section of the lung obtained on postmortem examination of case 1. Haematoxylin & eosin. (A) *Angiostrongylus vasorum* larvae within the lumen of a bronchus. ×20. (B) Adult *A vasorum* in a pulmonary artery. ×10

to this onset, the owners described a 12-hour period of bilateral dorsal strabismus, but no trauma or health problems were reported prior to the onset. The animal's vaccinations were up to date and, as in case 1, there had been no known exposure to rodenticides. Investigations (complete blood count and serum biochemical profile) performed by the referring veterinarian were all within normal limits. Abnormalities noted on physical examination included a focal intra-retinal haemorrhage in the left dorsolateral tapetal fundus. Neurological examination revealed a depressed mental status, normal posture and mild, left-sided hemiparesis. Hopping and placement testing revealed severe postural reaction deficits on the left thoracic and left pelvic limbs. Abnormalities on cranial nerve examination included absent menace response with normal pupillary light reflex on the left eye, decreased facial sensation on the left side and positional ventral strabismus on the right eye on dorsal flexion of the head. Segmental spinal cord reflexes were intact in all four limbs. The neuroanatomical diagnosis was consistent with a multifocal neurolocalisation (right forebrain and brainstem). Differentials were as documented for case 1.

A complete blood count and serum biochemistry profile were performed and found to be within reference ranges apart from a moderate thrombocytopenia of 100×10^9/litre (reference range 200 to 500×10^9/litre). Thoracic radiographs revealed a mixed bronchointerstitial lung pattern with multiple linear and ring markings considered abnormal for the age of the dog. There was mild right ventricular enlargement. Anaesthesia and an MRI scan of the brain were performed as for case 1. An irregular, globoid intra-axial mass 1·5 cm in diameter was present within the right temporal lobe white matter, causing a mild mass effect on the adjacent thalamus and right lateral ventricle. The mass had a mixed signal intensity on T2-weighted images with an irregular hyperintense centre surrounded by a hypointense rim and hyperintense perilesional oedema. On T1-weighted images, the mass was predominantly hyperintense with a narrow hypointense rim (Fig 2a). On gradient echo images, the lesion had a hyperintense centre with an irregular, very hypointense periphery (Fig 2b). Mild ring enhancement was detected on T1-weighted images after injection of contrast medium. A second smaller hypointense lesion within the white matter of the ventral aspect of the right occipital lobe was detected on gradient echo images. These image findings were considered consistent with subacute multifocal intracerebral haemorrhages, based on the hyperintensity seen on a T1-weighted image and hypointensity seen on gradient echo and T2-weighted images.

Differentials considered for this intracranial haemorrhage included primary (hypertensive) intracerebral haemorrhage and intracerebral haemorrhage following coagulopathies, vasculitis, intracranial tumour or rupture of a vascular malformation. Clotting profile revealed a markedly prolonged PT of 15·2 seconds, an APTT of 22 seconds and a TCT of 22·5 seconds. The dog's fibrin degradation products (FDP) were assessed and found to be over 1000 ng/ml (reference range <250 ng/ml). The differential diagnosis considered for this abnormal coagulation profile was similar to the one described in case 1. Chronic disseminated intravascular coagulation (DIC) was suspected based on the elevated FDP, prolongation of the clotting times and thrombocytopenia. A faecal sample examined by the Baermann technique was strongly positive for *Angiostrongylus* larvae.

The dog was prescribed a seven-day course of 50 mg/kg fenbendazole (Panacur 10 per cent liquid wormer; Hoechst) orally once daily. One week later, the coagulation profile was reported to be normal and the

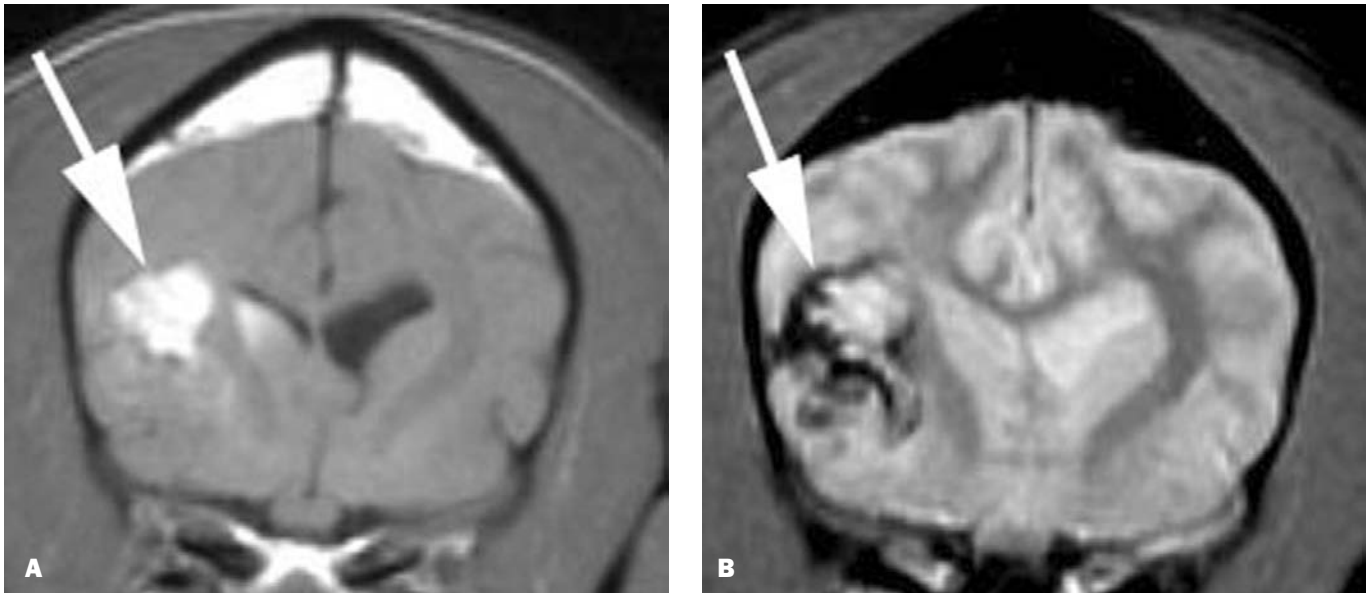

FIG 2. Transverse (A) T1-weighted fast spin echo and (B) T2*-gradient echo magnetic resonance images of the brain at the level of the optic chiasm of case 2. An ill-defined intra-axial mass (arrow), hyperintense in T1-weighted images with a narrow hypointense rim, is visible within the right temporal lobe. On gradient echo images, the same mass has a hyperintense centre with an irregular, very hypointense periphery

dog was clinically normal at the referring veterinarian's neurological examination. Repeat faecal analysis two weeks later was negative for *Angiostrongylus* larvae.

Case 3

A 14-month-old, neutered female Staffordshire bull terrier was presented for investigation of a 10-day history of intermittent ataxia on all four limbs, circling, lethargy, loss of vision and one episode of collapse two days before presentation. The owners noted no trauma or health problems prior to the onset. The animal's vaccinations were up to date and there had been no known exposure to rodenticides.

On presentation, the physical examination the dog was normal. On neurological examination, the dog was mentally dull. Posture and gait were normal. Postural reactions were abnormal on the left thoracic and left pelvic limbs. Spinal reflexes were normal on all four limbs. Cranial nerve examination revealed a decreased menace response in the left eye, absent menace response on the right eye and left nasal hypaesthesia. A positional right-sided rotatory nystagmus was noted on dorsal flexion of the head. No other cranial nerve abnormalities were noted. The neurological examination supported a multifocal forebrain and brainstem neurolocalisation. Differentials were as documented for case 1.

Complete blood count and serum biochemical profile were performed on admission. The complete blood count revealed a mature neutrophilia of $20{\cdot}1\times10^9$/litre (reference range 4 to 12×10^9/litre), mild anaemia with a reduced red blood cell count of $4{\cdot}8\times10^{12}$/litre (reference range 5·5 to $8{\cdot}5\times10^{12}$/litre) and a reduced haemoglobin concentration of 11·2 g/dl (reference range 12 to 18 g/dl) and severe thrombocytopenia of 39×10^9/litre. Serum biochemistry was within normal limits. Thoracic radiographs revealed an abnormal lung pattern with a generalised unstructured interstitial lung pattern with ill-defined, diffuse increased opacity and a small volume of pleural fluid. A moderate bronchial pattern was superimposed over the interstitial lung pattern (Fig 3). The heart and pulmonary vasculature appeared normal.

Anaesthesia and an MRI scan of the brain were performed as for case 1. The

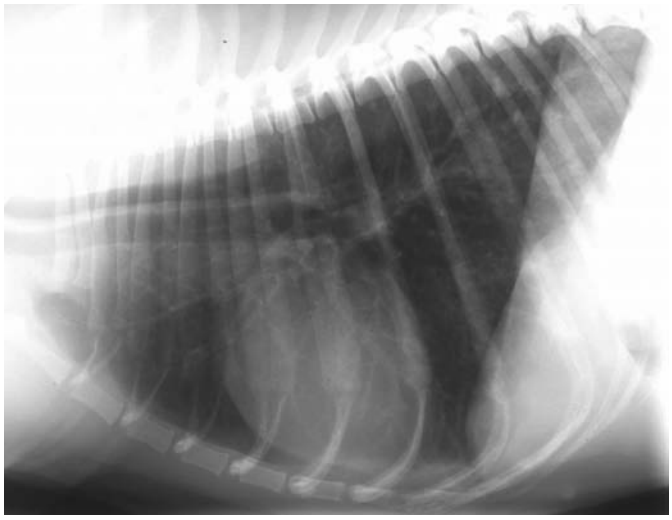

FIG 3. Lateral thoracic radiographs obtained from case 3. The main feature is a grossly abnormal lung pattern with a generalised unstructured interstitial lung pattern with ill-defined, diffuse increased opacity. Superimposed over the interstitial lung pattern is a moderate bronchial pattern

MRI scans showed multifocal areas of mixed signal with mass effect within the cerebral cortex of the left frontal lobe and right parietal lobe. The lesions appeared irregular and ill-defined, with a mixed low and high signal intensity on T2- and T1-weighted images and uniform hypointensity on gradient echo images suggestive of subacute haemorrhage. The gradient echo images showed, in addition to the two larger areas of haemorrhage, multiple small pinpoint areas of haemorrhage throughout the forebrain. Extensive perilesional oedema extended throughout the right internal capsule. Intraventricular haemorrhage was present and was characterised by increased signal intensity of T1-weighted and fluid attenuated inversion recovery (FLAIR) images and small areas of decreased signal intensity on T2-weighted and gradient echo images in the dependent portion of the right lateral ventricle.

The differential diagnosis considered for this intracranial haemorrhage was similar to the one described in case 2. A clotting profile revealed a markedly prolonged PT of 40·0 seconds, an APTT of 37·5 seconds and a TCT of longer than 120 seconds. The dog's FDPs were assessed and found to be over 1000 ng/ml. The differentials considered for this abnormal coagulation profile were similar to those described in case 1 and 2. Chronic DIC was suspected based on the elevated FDP, prolongation of the clotting times and thrombocytopenia.

The dog was immediately given an intravenous plasma transfusion at a dose of 2 ml/kg/hour (total dose 300 ml). A faecal sample examined by the Baermann technique was strongly positive for *Angiostrongylus* larvae. A seven-day course of 50 mg/kg fenbendazole orally once daily was prescribed. A repeated clotting profile 24 hours after the plasma transfusion revealed a TCT of 10 seconds. The platelet count, PT and APTT had returned to normal. At the time of discharge, five days after admission, the neurological examination and clotting profile were normal. Repeat faecal analysis two weeks after fenbendazole treatment was negative for *Angiostrongylus* larvae. Repeat neurological examination 10 weeks later was normal.

DISCUSSION

Non-traumatic intracerebral haemorrhage is bleeding into the parenchyma of the brain that may extend into the ventricles and, in rare cases, the subarachnoid space. Depending on the underlying cause of bleeding, intracerebral haemorrhage can be classified as either primary or secondary (Qureshi and others 2001). Primary haemorrhage originates from the spontaneous rupture of small vessels damaged predominantly by chronic hypertension and degenerative changes in cerebral arteries such as amyloid angiopathy (Foulkes and others 1988).

In contrast to the high incidence in humans, intracerebral haemorrhage resulting from spontaneous rupture of vessels is considered rare in dogs (Fankhauser and others 1965). Secondary haemorrhage has been reported in dogs in association with various causes such as rupture of congenital vascular abnormalities (Fankhauser and others 1965, Thomas and others 1997), haemorrhage into primary and secondary brain tumours (Fankhauser and others 1965, Joseph and others 1988), inflammatory disease of the arteries and veins or intravascular lymphoma (malignant angioendotheliomatosis) (McDonough and others 2002), brain infarction (haemorrhagic infarction) (Tidwell and others 1994) or impaired coagulation (Joseph and others 1988, Dunn and others 1995). Clinical studies of cerebrovascular disease such as intracerebral haemorrhage in dogs consist mostly of isolated case reports, and large-scale studies on this subject are currently lacking.

MRI findings in these three cases were considered consistent with cerebral haemorrhage mainly on a combination of gradient echo, T1- and T2-weighted signal intensities of the lesion, mass effect, location of the lesion and acute to subacute onset of the clinical signs. The two most important biophysical properties in the generation of signal intensity patterns on MRI scans seen in evolving intracranial haematomas are the paramagnetic effects of iron associated with the changing oxygenation states of haemoglobin and the integrity of red blood cell membranes that, when intact, compartmentalise the paramagnetic iron (Atlas and Thulborn 2002). However, the signal intensity of intracranial haemorrhage is also influenced by several intrinsic (time from ictus, source, size and location of haemorrhage) and extrinsic (pulse sequence and field strength) factors (Zimmerman and others 1988). The causes of these intrinsic and extrinsic variations in haematoma intensity are difficult to evaluate with clinical studies, since it is frequently impossible to precisely ascertain the interval between haemorrhage and MRI scanning.

Gradient echo sequences have been proven to be the most accurate of all of the MRI pulse sequences, and more accurate than computed tomography, in predicting the extent of haemorrhage on pathological examination in a dog model (Weingarten and others 1991). Compared with other sequences, gradient echo scans readily demonstrate detectable hypointensity, regardless of the time from ictus, the source and location of haemorrhage, or the field strength (Weingarten and others 1991). Hyperintensities on gradient echo images are, however, not specific for haemorrhage and may also be seen with calcification, air, iron, foreign bodies and melanin (Atlas and Thulborn 2002). Air, calcification and foreign bodies would also normally be hypointense on all the pulse sequences (Atlas and Thulborn 2002).

Marked hyperintensity on T1-weighted scans was observed in two of the three cases presented here. This hyperintensity in T1-weighted scans is present whenever methaemoglobin exists and thus is seen mainly during the first few days of the disease (early subacute haematoma) (Atlas and Thulborn 2002). Hyperintensity within the brain parenchyma in

T1-weighted scans is abnormal. In addition to haemorrhage, it may be seen with fat, flow artefacts, very high non-paramagnetic protein content, intra-tumoural melanin, contrast agent and calcification. The pattern of signal intensity observed on the T2-weighted scans appeared variable between cases.

The demonstration of high levels of FDPs, decreased numbers of platelets and the prolongation of the clotting times in the three presented cases demonstrated that the coagulopathy was associated with DIC. This common consumptive coagulopathy results from a wide variety of causes including infection, inflammation, neoplasia and parasitism (Welles 1996). The basic process depends on the release of thromboplastic substances from damaged tissue, resulting in the activation of the coagulation process and the formation of fibrin, in the course of which clotting factors and platelets are consumed (Greene 1975). As a pathophysiological mechanism, DIC is seldom primary. It is usually an intermediary or secondary condition that accompanies various pathological conditions and virtually any mechanism that produces tissue damage can result in the release of tissue thromboplastins into the circulation (Slappendel 1988).

The essential pathological change in DIC is the occurrence of widespread fibrin thrombi in small vessels, resulting in numerous small infarctions of many organs, including the brain. The main reason for the haemorrhage is the consumption of platelets and various clotting factors that occurs during fibrin formation (Adams and others 1997).

Pathology and clinical reports have indicated that there might be a possible relationship between *A vasorum* infection and host coagulation abnormalities (Schelling and others 1986, Ramsey and others 1996, Gould and McInnes 1999). In addition, thrombocytopenia (in the absence of prolongation of clotting times) has been associated with this parasite and attributed to the presence of antiplatelet antibodies (Gould and McInnes 1999). Coagulation abnormalities in dogs infected with *A vasorum* have been attributed to excessive intravascular coagulation in a naturally occurring case of angiostrongylosis (Ramsey and others 1996).

The role of coagulation defects in the pathogenesis of angiostrongylosis has been described by Schelling and others (1986) in an experimentally infected dog with *A vasorum*. In addition, Caruso and Prestwood (1988) demonstrated deposits of immunoglobulins, complements and fibrinogen in the alveoli, bronchial lining and tunicae intima of blood vessels in lungs of dogs experimentally infected with *A vasorum*. It has been suggested that the intrinsic pathway might have been activated by complement-mediated damage to vascular endothelium resulting in potential collagen exposure to the blood as well as by the presence of vascular luminal immune deposits. The extrinsic pathway might have been activated by the release of thromboplastin from damaged tissue, such as that which occurs with complement fixation (Caruso and Prestwood 1988). The continued presence of fibrinogen as the predominant protein deposited suggested a continuation of intravascular coagulation (Caruso and Prestwood 1988). The activation of the clotting cascade produces an intravascular consumptive coagulopathy characterised by thrombocytopenia with prolongation of APTT and PT, as observed in the three cases presented in this report.

The management of chronic DIC is aimed primarily at identifying and correcting the underlying cause. As the major cause of the bleeding is a depletion of clotting factors, the replacement of these factors by the administration of fresh plasma is the most important aspect of therapy. This allowed sufficient time for the diagnosis of the underlying cause to be made in case 3. Oral treatment with 50 mg/kg fenbendazole once or twice daily for one week or subcutaneous treatment with 0·2 mg/kg ivermectin twice at an interval of approximately one week have both been advocated to treat angiostrongylosis (Bolt and others 1994). Compared with levamisole, fenbendazole is thought to have a much higher therapeutic index and to exert its effect more slowly, and is therefore proposed to reduce the risk of side effects or anaphylaxis due to the sudden release of large amounts of immunogenic worm fragment antigen (Drade and Guirand 1977).

A definitive diagnosis of *A vasorum* infection can be difficult to establish. Although not specific, radiographic evidence consists of diffuse bronchial and interstitial patterns, small areas of alveolar pattern and occasionally nodular interstitial infiltrates. Lung disease is most severe radiographically at seven to 10 weeks post-infection when the alveolar pattern predominates and is associated with haemorrhage into the alveolar spaces during the onset of patency (Mahaffey and others 1981). In this respect, the pattern may resemble that observed in cases of rodenticide poisoning. However, rodenticide poisoning without a known history of exposure is thought to be rare. The historical, clinical and radiographic findings may lead to angiostrongylosis being included in the differential diagnosis of a coagulopathy as observed in the dogs presented here. Positive identification of *A vasorum* larvae in bronchoalveolar washes or faecal samples is the best method of diagnosis when the infection is patent (Bolt and others 1994).

Finally, *A vasorum* has been reported in the UK in Cornwall (Jones and others 1980, Simpson and Neal 1982, Martin 1989, Martin and Neal 1992, Martin and others 1993), south Wales (Patteson and others 1987), south east England (Cobb and Fisher 1990) and more recently Surrey (Chapman 2004). However, it seems likely from the increasingly wide variety of areas from which cases are being reported that the geographical range of the parasite within the UK is either more widespread than previously supposed or that its geographical distribution has spread in recent years. The dog in case 1 had been born and lived in the west midlands of England with no travel history to endemic areas. On

questioning the owners, the dog in case 2 had recently spent time in Bridport, Dorset, a known endemic area. The dog in case 3 had also spent regular time over the previous few months in Surrey.

Conclusions

This case series indicates that *A vasorum* infection should be considered as a possible aetiology of intracranial haemorrhage in dogs.

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References

ADAMS, R. D., VICTOR, M. & ROPPER, A. H. (1997) Cerebrovascular disease. In: Principles of Neurology. 6th edn. Eds R. D. Adams, M. Victor and A. H. Ropper. McGraw-Hill, New York. pp 777-873

ATLAS, S. W. & THULBORN, K. R. (2002) Intracranial hemorrhage. In: Magnetic Resonance Imaging of the Brain. 3rd edn. Ed W. Scott. Lippincott Williams & Wilkins, Philadelphia. pp 773-832

BOLT, G., MONRAD, J., KOCH, J. & JENSEN, A. L. (1994) Canine angiostrongylosis: a review. *Veterinary Record* **135**, 447-452

CARUSO, J. P. & PRESTWOOD, A. K. (1988) Immunopathogenesis of canine angiostrongylosis: pulmonary effect of infection. *Comparative Immunology, Microbiology and Infectious Disease* **11**, 85-92

CHAPMAN, P. S., BOAG, A. K., GUITIAN, J. & BOSWOOD, A. (2004) *Angiostrongylus vasorum* infection in 23 dogs (1999-2002). *Journal of Small Animal Practice* **45**, 435-440

COBB, M. A. & FISHER, M. A. (1990) *Angiostrongylus vasorum*: transmission in south east England. *Veterinary Record* **126**, 529

Dodd, K. (1973) *Angiostrongylus vasorum* infestation in greyhound kennels. *Veterinary Record* **92**, 195-197

DRADE, T. & GUIRAND, C. (1977) Diagnosis and treatment of cardiopulmonary angiostrongylosis. Proceedings of the 6th World Congress of the WSAVA. Royal Netherlands Veterinary Association, Amsterdam. pp 141-142

DUNN, K. J., NICHOLLS, P. K., DUNN, J. K. & HERRTAGE, M. E. (1995) Intracranial haemorrhage in a dobermann puppy with von Willebrand's disease. *Veterinary Record* **136**, 635-636

FANKHAUSER, R., LUGINBUHL, H. & MCGRATH, J. T. (1965) Cerebrovascular disease in various animal species. *Annals of the New York Academy of Sciences* **127**, 817-859

FOULKES, M. A., WOLF, P. A., PRICE, T. R., MOHR, J. P. & HIER, D. B. (1988) The Stroke Data Bank: design, methods, and baseline characteristics. *Stroke* **19**, 547-554

GOULD, S. M. & MCINNES, E. L. (1999) Immune-mediated thrombocytopenia associated with *Angiostrongylus vasorum* infection in a dog. *Journal of Small Animal Practice* **40**, 227-232

GREENE, C. E. (1975) Disseminated intravascular coagulation in the dog: a review. *Journal of the American Animal Hospital Association* **11**, 675-687

JONES, G. W., NEALE, C. & TURNER, G. R. (1980) *Angiostrongylus vasorum* infection in dogs in Cornwall. *Veterinary Record* **106**, 83

JOSEPH, R. J., GREENLEE, P. G., CARILLO, J. M. & KAY, W. J. (1988) Canine cerebrovascular disease: clinical and pathological findings in 17 cases. *Journal of the American Animal Hospital Association* **24**, 569-576

KING, M. C. A., GROSE, R. M. R. & STARTUP, G. (1994) *Angiostrongylus vasorum* in the anterior chamber of a dog's eye. *Journal of Small Animal Practice* **35,** 326-328

MCDONOUGH, S. P., VAN WINKLE, T. J., VALENTINE, B. A., VAN GESSEL, Y. A. & SUMMERS, B. A. (2002) Clinicopathological and immunophenotypical features of canine intravascular lymphoma (malignant angioendotheliomatosis). *Journal of Comparative Pathology* **126**, 277-288

MAHAFFEY, M. B., LOSONSKY, J. M., PRESTWOOD, A. K., MAHAFFEY, E. A. & LEWIS, R. E. (1981) Experimental canine angiostrongylosis. II. Radiographic manifestations. *Journal of the American Animal Hospital Association* **17**, 499-502

MARTIN, M. W. S. (1989) Heartworm death in a young dog. *Veterinary Record* **124**, 470

MARTIN, M. W. S., ASHTON, G., SIMPSON, V. R. & NEAL, C. (1993) Angiostrongylosis in Cornwall: clinical presentation in eight cases. *Journal of Small Animal Practice* **34**, 20-25

MARTIN, M. W. S. & NEAL, C. (1992) Distribution of angiostrongylosis in Cornwall. *Journal of Small Animal Practice* **33**, 327-330

MURDOCH, D. B. (1984) Heartworm in the United Kingdom. *Journal of Small Animal Practice* **25**, 299-305

PATTESON, M. W., GIBBS, C., WOTTON, P. R. & DAY, M. J. (1993) *Angiostrongylus vasorum* infection in seven dogs. *Veterinary Record* **133**, 565-570

PATTESON, M. W., WOTTON, P. R., LUCKE, V. M., WRIGHT, A. I. & GIBBS, C. (1987) *Angiostrongylus vasorum* in a terrier. *Veterinary Record* **120**, 349

PERRY, A. W., HERTLING, R. & KENNEDY, M. J. (1991) Angiostrongylosis with disseminated larval infection associated with signs of ocular and nervous disease in an imported dog. *Canadian Veterinary Journal* **32**, 430-431

PRESTWOOD, A. K., GREENE, C. E., MAHAFFEY, E. A. & BURGESS, D. E. (1981) Experimental canine angiostrongylosis. I. Pathological manifestations. *Journal of the American Animal Hospital Association* **17**, 491-497

QURESHI, A. I., TUHRIM, S., BRODERICK, J. P., BATJER, H. H., HONDO, H. & HANLEY, D. F. (2001) Spontaneous intracerebral hemorrhage. *New England Journal of Medicine* **344**, 1450-1460

RAMSEY, I. K, LITTLEWOOD, J., DUNN, J. K. & HERRTAGE, M. E. (1996) Role of chronic disseminated intravascular coagulation in a case of canine angiostrongylosis. *Veterinary Record* **138**, 360-363

SCHELLING, C. G., GREENE, C. E., PRESTWOOD, A. K. & TSANG, V. C. W. (1986) Coagulation abnormalities associated with acute *Angiostrongylus vasorum* infection in dogs. *American Journal of Veterinary Research* **47**, 2669-2673

SIMPSON, V. R. & NEAL, C. (1982) *Angiostrongylus vasorum* infection in dogs and slugs. *Veterinary Record* **111**, 303-304

SLAPPENDEL, R. J. (1988) Disseminated intravascular coagulation. *Veterinary Clinics of North America: Small Animal Practice* **18**, 169-184

THOMAS, W. B., ADAMS, W., MCGAVIN, M. D. & GOMPF, R. E. (1997) Magnetic resonance imaging appearance of intracranial haemorrhage secondary to cerebral vascular malformation in a dog. *Veterinary Radiology & Ultrasound* **38**, 371-375

TIDWELL, A. S., MAHONY, O. M., MOORE, R. P. & FITZMAURICE, S. N. (1994) Computed tomography of an acute hemorrhagic cerebral infarct in a dog. *Veterinary Radiology & Ultrasound* **35**, 290-296

WEINGARTEN, K., ZIMMERMAN, R. D., DEO-NARINE, V., MARKISZ, J., CAHILL, P. T. & DECK, M. C. F. (1991) MR imaging of acute intracranial hemorrhage: findings on sequential spin-echo and gradient echo images in a dog model. *American Journal of Neuroradiology* **12**, 457-467

WELLES, E. G. (1996) Antithrombotic and fibrinolytic factors: a review. *Veterinary Clinics of North America: Small Animal Practice* **18**, 169-184

ZIMMERMAN, R. D., HEIER, L. A., SNOW, R. B., LIU, D. P. C., KELLY, A. B. & DECK, M. D. F. (1988) Acute intracranial hemorrhage: intensity changes on sequential MR scans at 0.5T. *American Journal of Neuroradiology* **9**, 47-57