Review of idiopathic eosinophilic meningitis in dogs and cats, with a detailed description of two recent cases in dogs

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ABSTRACT

Eosinophilic meningoencephalitis (EME) has been described in various species of animals and in humans. In dogs it has been associated with protozoal infections, cuterebral myiasis and various other aetiologies. Ten cases of idiopathic eosinophilic meningoencephalitis have been reported in dogs and one in a cat where the origin was uncertain or unknown. The dogs were all males, of various breeds but with a predominance of Golden Retrievers and Rottweilers; they generally had a young age of onset. Two cases with no apparent underlying aetiology were diagnosed on *post mortem* examination. The 18-month-old, male Boerboel presented with sudden onset of cerebellar ataxia, as well as various asymmetrical cranial nerve deficits of 2 weeks' duration and without progression. Haematology revealed a peripheral eosinophilia. Necropsy showed extreme generalised congestion especially of the meninges and blood smear and histological sections of various tissues showed intravascular erythrocyte fragmentation with the formation of microcytes. Histopathology revealed severe diffuse cerebrocortical subarachnoidal meningitis and submeningeal encephalitis, the exudate containing variable numbers of eosinophils together with neutrophils and mononuclear cells. There was also deeper white matter and hippocampal multifocal perivascular mononuclear encephalitis and multifocal periventricular malacia, gliosis and phagocytosis of white matter. The cerebellum, brain stem and spinal cord showed only mild multifocal oedema or scattered occasional axon and myelin degeneration respectively, with no inflammation. Immunohistochemical staining of central nervous tissue for *Toxoplasma gondii* failed to show any antigen in the central nervous tissue. Ultrastructure of a single submeningeal suspected parasitic cyst showed it to be chromatin clumping within a neuron nucleus indicating karyorrhexis. Gram stain provided no evidence of an aetiological agent. The 3-year-old Beagle bitch had a Caesarian section after developing a non-responsive inertia 8 days prior to presentation. This animal's clinical signs included *status epilepticus* seizures unrelated to hypocalcaemia and warranted induction of a barbiturate coma. She died 4 hours later. *Post mortem* and histopathological findings in the brain were almost identical to those of the Boerboel and she also showed histological evidence of recent active intravascular haemolysis with microcyte formation. Rabies, distemper and *Neospora caninum* immunohistochemical stains were negative in the brains of both dogs. Immunohistochemical staining of the cerebral and meningeal exudates of the Beagle for T- and B-lymphocyte (CD3 and CD79a) markers showed a predominance of T-lymphocytes with fewer scattered B lymphocytes. A possible allergic response to amoxicillin/clavulanate is considered, as this appeared to be the only feature common to the recent history of both animals. An overview of EME in humans, dogs and cats is given and the previously published cases of idiopathic EME in dogs and the single published cat case are briefly reviewed.

Key words: Beagle, Boerboel, cats, dogs, drug-induced allergy, eosinophilic meningoencephalitis, histopathology, idiopathic, intravascular haemolysis, review.

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INTRODUCTION

Eosinophilic meningoencephalitis (EME) is diagnosed in humans and animals when neurological disease is associated with eosinophilic pleocytosis, which is the presence of increased numbers of eosinophils in the cerebrospinal fluid (CSF). In a series of 93 dogs with neurological disease, eosinophilic pleocytosis was found in single cases of cryptococcosis (80% of the white cells counted in the CSF were eosinophils), toxoplasmosis (2.5 %), infarction of the caudate nucleus (6 %), cerebrocortical infarction (7 %), cerebral lymphosarcoma (1.5 %), in 4 dogs with granulomatous encephalitis (23.5 %), and in 5 dogs with tumours of the spinal and epidural tissues (1.4 %)[32]. Meningoencephalitis with eosinophilic pleocytosis has also been recognised in aberrant migration of *Angiostrongylus cantonensis* (the lung-worm of rats found in South-east Asia and various islands including Hawaii) in dogs, protothecal meningoencephalitis, distemper virus, rabies virus and bacterial infections[9,27,28,31]. The largest numbers of eosinophils were found in cryptococcal and protothecal meningoencephalitis[27,31,32]. EME has additionally been reported in 1 of 2 cats and 1 dog with intracranial cuterebral larval migration[6,19]. There is a report of a cat with neurological signs and eosinophilic pleocytosis that recovered after a therapeutic trial of levamisole, leading to a possible differential diagnosis of cuterebriasis[6].

Toxoplasma gondii or *Neospora caninum* were suggested as causes of EME in 2 dogs of a series of 8 canine cases in a Canadian study[28]. In 1 of these 2 dogs, apicomplexan-like cysts were visualised in the brain and other tissues but no further characterisation of the cyst was done; the other case had a granulomatous meningoencephalitis suggesting a protozoan infection but no organisms were

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found. These 2 cases, for this reason, have been excluded as cases of idiopathic EME for the purposes of this review.

The underlying aetiologies of EME in humans include *Angiostrongylus cantonensis, Gnanthostoma spinigerum,* cysticercosis, schistosomiasis, paragonimiasis, fascioliasis and *Toxocara canis*[12,27,34]. Tuberculosis, syphilis, coccidiodomycosis and lymphoma involving the meninges can also occasionally cause human CSF eosinophilia[34]. Less frequently encountered non-infectious human eosinophilic pleocytoses have been reported with pneumoencephalography, myelography, intrathecal administration of penicillin and radioiodinated serum albumin, central nervous system (CNS) rubber surgical shunts, multiple sclerosis and subarachnoid haemorrhages[3,7,12,18,29].

If no aetiological agent is found in cases of EME, the term 'idiopathic' has been adopted. Idiopathic EME is a rare neurological condition of unknown origin with 10 cases in dogs and 1 in a cat having been reported to date[2,26,27,28]. These cases are reviewed in the form of discussion and roughly in the sequence of breed, age and gender incidence; clinical signs; haematological findings; CSF cytology and chemistry findings; tests performed for possible aetiologies; case progression, treatment, maintenance and outcome; and necropsy and histological findings, if performed. The Siberian husky is discussed as a separate entity owing to the lack of similarities to the other canine cases[28].

REVIEW OF REPORTED CASES

Incidence

The 10 dogs reported with idiopathic EME were all males and comprised 6 dogs from Canada (3 Golden retrievers, a Yorkshire terrier, a Siberian husky and a mixed-breed dog) ranging in age from 14 weeks to 5.5 years; 3 Rottweilers aged 12, 15 and 17 months from New Zealand; and a 6-month-old Maremma shepherd dog from Italy, in which clinical signs had begun at 2 months of age[2,26,28].

Clinical signs

Clinical signs reported in the dogs included depression, somnolence, ataxia, circling, hindlimb proprioception deficits, collapse, neck pain, reduced menace reflex, cerebral blindness, loss of gag reflex, behavioural abnormalities, house soiling and seizures, with slow progression ranging from 1 week to 12 months[2,26,28].

The cat with idiopathic EME was a 6-year-old male domestic shorthair admitted to the Michigan State University Veterinary Clinical Centre[27]. It had presented with a history of excessive drooling and grooming, facial pawing, and vocalisation. Three weeks earlier a similar episode of hypersalivation and facial pawing had occurred but spontaneously resolved after 20 minutes. The cat was mainly house-bound but occasionally free to roam in the neighbourhood where it was known to have contact with rabbits and skunks, but with no known exposure to toxic plants or chemicals, and it had received no medications[27]. At presentation it had congested oral mucosa and forepaws, a stiff posture with claws extended, unilateral muscle fasciculations of the face and frantic pawing at the mouth. It was tachypnoeic (160 breaths/minute) and rectal temperature was 38.8 °C. By the second day there was disorientation, inability to stand, depressed placing and tactile responses and apparent loss of vision and hearing; there were no ocular lesions. Occasional brief tonic-clonic seizures occurred during periodic salivation, focal facial motor seizures and pawing episodes.

Haematological analyses

Haematological analyses of the dogs showed that peripheral white cell counts were within reference range for all dogs except the 15-month-old Rottweiller (23.8×10^9/ ; reference interval 6.0–15.0 $\times$ 10^9/), from the New Zealand case report. Furthermore, in dogs, peripheral blood eosinophilia was not always present and therefore not a reliable indicator of EME (peripheral eosinophils in these animals ranged from 0.14–10.47 $\times$ 10^9/ ; reference interval 0.0–1.9 $\times$ 10^9/)[2,28]. The Maremma sheepdog had shown eosinophilia on haemogram of 2.82 $\times$ 10^9/ (reference interval 0.29–1.3 $\times$ 10^9/) with a white cell count of 14.1 $\times$ 10^9/ (reference interval 6.0–16.0 x10^9/)[26].

The cat had shown a mild stress-type leukogram characterised by neutrophilia without left shift and lymphopaenia[27].

CSF analyses

CSF analysis was declined by the owner of the Maremma sheepdog[26]. The degree of peripheral eosinophilia in the other 9 dogs also did not in all cases correlate with the eosinophilic pleocytosis in the CSF; CSF total nucleated cell count (TNCC) ranged from 11–5500 cells/μ (reference interval 0–5, which should all be mononuclear). The percentage of CSF eosinophils varied from 5–98 % (reference 0 %)[2,28]. CSF protein levels ranged from normal (10–25 mg/d) in 2 dogs, to mildly elevated (38 mg/d) to markedly elevated (1430 mg/d) in the other 7 dogs[2,28].

CSF from the cat showed mild pleocytosis (TNCC of 17 cells/μ ; reference range 0–8 cells/μ) with 81 % eosinophils and mildly elevated protein. No aetiological agent was found in the cytological preparation[27].

Aetiological tests

Only 1 dog (the Siberian husky of the Canadian series) was positive for *Toxocara canis* on faecal flotation[28]. All of the previously reported cases of canine idiopathic EME tested serologically negative for toxoplasmosis[2,26,28]. The year-old Rottweiler was also negative for *Neospora caninum* antibodies in serum and CSF. The Maremma sheepdog tested negative on faecal flotation for parasite eggs and on serological assays for *Toxoplasma gondii, Neospora caninum, Dirofilaria immitis* and canine distemper virus infections. In all cases the most recent vaccination, specifically rabies vaccination, was administered more than 3 weeks in the case of the 4-month-old Golden retriever pup and in the others, more than 2 months prior to presentation[16].

The cat had tested negative for feline leukaemia virus, toxoplasmosis, *Dirofilaria* sp. and cryptococcosis. Feline infectious peritonitis was unlikely considering the history and recovery; a type 1 hypersensitivity reaction was suspected[27]. Urinalysis had 2+ proteinuria (100 mg/d) and faecal flotation was negative.

Case progression, treatment, maintenance and outcome

Of the 6 canine cases of idiopathic EME from the Canadian report, 4 survived and had variable to good responses to either chloramphenicol alone (1 case) and/or dexamethasone treatment of varying durations and dosages. Two of the 4 survivors had to be maintained on anticonvulsive therapy (phenobarbitone) as well as the dexamethasone in an attempt to control seizures after the presenting episodes. One case, the 4-month-old Golden retriever, responded to a 2-week course of chloramphenicol alone at 500 mg/kg 3 times daily; 5 years later it was neurologically normal apart from a residual hyporeflexia of the right patellar reflex[28]. Of the 3 New Zealand cases, 2 of the 3 cases survived; the 15-month-old Rottweiller had no further neurological episodes for the 4-month follow-up period and the 1-year-old Rottweiler had residual episodic seizures and circling and was maintained on prednisolone and anticonvulsant therapy[2]. The Maremma sheepdog from Italy was euthanased after the magnetic resonance imaging revealed diffuse necrosis or atrophy of the cerebral cortical grey matter[26]. Treatment had not been attempted.

The cat with EME had a good response to a diminishing course of dexamethasone

over approximately 4 weeks[27]. When checked 1 month after the episode it was found to have only a slightly decreased menace reflex in the left eye. At a 15 month follow-up check it had shown no relapse and there were no residual neurological changes.

Necropsies and histopathology

Post mortem examinations were conducted on 4 of the 10 dogs; the 18-month-old Golden retriever and 3.5-year-old Siberian husky from the Canadian study, the 17-month-old Rottweiler from the New Zealand study and the 6-month-old Maremma sheepdog from Italy[2,26,28]. The owner of the 4-year-old Canadian series Golden retriever refused necropsy. In the younger Golden retriever, gross necropsy examination showed bilateral cerebral cortical atrophy with opaque white exudate in sulci and the pyriform lobe meninges appearing green. Histological study revealed severe eosinophilic and granulomatous meningitis especially involving the cerebral hemispheres, with vacuolated underlying neuronal parenchyma. There was neuronal pyknosis, variable demyelination of cerebral white matter and diffuse gliosis and astrocytosis of cerebral grey matter, with perivascular cuffing being confined to the superficial surface of the cortices[28].

The only abnormal *post mortem* findings in the Rottweiler were found in the brain[2], which had thickened and green-discoloured meninges. The spinal cord was not examined. Histopathology of the brain showed a moderately severe, diffuse meningeal eosinophil infiltrate, which extended slightly into the superficial cortical grey matter – this 0.1-mm superficial layer appeared spongy with prominent blood vessels and scattered hypertrophic astrocytes.

A *post mortem* performed after euthanasia of the Maremma sheepdog and subsequent histopathological examination revealed subarachnoid spaces expanded by a severe inflammatory infiltration composed mainly of eosinophils (stained for peroxidase), macrophages, many of which showed phagocytosis of eosinophils, and moderate numbers of lymphocytes and plasma cells[26]. Inflammatory infiltrates relatively spared the cerebral cortex apart from the superficial layer and subpial blood vessels, which showed perivascular cuffing. The inflammation was accompanied by spongiosis of the parenchyma with rarefaction and astrocytosis with gitter cells and there was also neuronal necrosis and loss in these superficial cortical regions. Axonal necrosis and demyelination were seen in the corticospinal tracts of the mid-brain and pyramids. No aetiological agents were found and no significant changes were observed in other regions of the CNS.

The Siberian husky deserves mention as an entity alone since he was an unusual case in all respects compared with the other cases reported[28]. His dam had had a history of seizures beginning at the age of 4 years, and he had shown 8–10 seizures over the 6 months prior to admission, at which he appeared clinically normal. The dog was dewormed and vaccinated 6 months prior to admission. On haematology there was peripheral eosinophilia ($3.32 \times 10^9/$) and faecal analysis was positive for *Toxacara canis.* CSF had a total nucleated cell count (TNCC) of 15 cells/μ (reference interval is 0–5cells/μ) of which 50 % were eosinophils, and CSF protein was normal. Treatment included phenobarbitone (3 mg/kg twice daily) and dexamethasone (0.25 mg/kg 3 times daily) with no further seizures in the next 4 weeks. At this time the dog was considered normal apart from weight gain and lethargy, and CSF TNCC was normal (2 cells/μ), but 55 % of these were still eosinophils. Three months later, while still on treatment, the episodic seizures recurred with an interval of 3 weeks and CSF TNCC was 15 cells/μ with 10 % eosinophils. The seizures gradually worsened over the next 10 months and an additional 4 more CSF analyses were performed and found to be normal. Corticosteroid treatment was withdrawn after 14 months due to side-effects. Clonazepam was added to the barbiturate antiepileptic therapy but did not control weekly seizures and the dog was subsequently euthanased. Necropsy of this Siberian husky showed neither gross nor microscopic brain lesions[28].

CASE HISTORIES

The 1st case study describes the history, haematology, clinical signs, *post mortem* and histopathology findings in a young, male Boerboel diagnosed with EME and with no apparent underlying aetiology. The 2nd case describes the clinical signs, treatment, blood chemistry, *post mortem* and histopathological findings in a post-whelping 3-year-old beagle bitch diagnosed as EME histopathologically after necropsy. To the best of our knowledge these are the first cases of idiopathic eosinophilic meningoencephalitis reported in dogs in Africa and the 1st case reported in a bitch.

Case history 1

An 18-month-old, intact male Boerboel was referred to the Department of Small Animal Medicine at the Onderstepoort Veterinary Animal Hospital with the chief complaint of sudden onset of ataxia of 2 weeks duration, and without progression. He had completed a 10-day course of amoxycillin-clavulanate (Synulox, Pfizer Laboratories, Sandton) for suspected cystitis diagnosed on urine sediment and history 20 days before he was euthanased. Therefore the clinical signs had begun approximately 6 days after the start of the antibiotic course. At the start of the course he was also routinely dewormed with a praziquantal, febantel, pyrantal embonate combination (Drontal, Bayer Animal Health Division, Isando) by the referring veterinarian. According to the owner and referring veterinarian, the dog-trainer had administered the rabies vaccination at the age of 6 months but no certification was issued. Other vaccines had been given when the dog was a puppy but also not by a veterinarian.

The owner described the dog as bright and alert, but reluctant to walk, often just collapsing into a sitting position or falling over, to no particular side. The dog was no longer able to lift his hind limb during urination, and easily slipped on smooth surfaces. During meals, food would continuously fall from his mouth, despite normal mastication.

A blood smear from the patient showed eosinophilia. A complete blood count confirmed moderate eosinophilia ($3.98 \times 10^9/$; reference interval $0.14–2.46 \times 10^9/$). Faecal flotations on presentation and at necropsy did not detect any helminth ova.

The neurological examination showed severe ataxia with the patient displaying a goose-stepping gait. Conscious proprioception was present, but unconscious proprioception was absent, with obvious crossing-over of the limbs when the patient moved in a circle. Hemi-stand and hopping reflexes were abnormal bilaterally but worse on the right. The dog tripped over most objects in an obstacle course, there was lack of left peripheral vision on the cotton-ball fixating reflex, but normal right peripheral vision, and the menace reflex was absent. The direct and consensual pupillary light reflexes were normal. On cranial nerve examination, there was no gag-reflex and when offered food, the patient would repeatedly overshoot the bowl, and oropharyngeal dysphagia was evident as often food fell from his mouth when attempting to swallow.

The retina was examined by an ophthalmologist and found to be within normal limits. The option of further investigation, including cerebrospinal fluid collection for cytology, viral and protozoal serology, and possible magnetic resonance imaging study of the brain, was declined by the owner who subsequently elected humane

euthanasia. The carcass was submitted for *post mortem* examination.

Necropsy findings

On *post mortem* examination the dog was found to be in good body condition. Examination of a peripheral blood smear taken from an ear and stained with Diff-Quik stain (Kyro-Quick®, Kyron Laboratories, Benrose) showed numerous irregularly sized and shaped fragments of red cells (schistocytes) towards the head of the smear, an increase in eosinophils and most neutrophils had cytoplasm filled by varying-sized clear vacuoles and some with phagocytosis of fragments of fractured red cells (Fig. 1); occasional neutrophils contained intracytoplasmic amorphous amphophilic material and some showed necrosis and fragmentation of their nuclei. A few immature neutrophils and active monocytes were also seen. Some of the eosinophils had degranulation of varying numbers of their cytoplasmic granules. Single platelets of varying sizes were scattered along the feathered edge.

The most significant macroscopic finding was marked generalised congestion of the carcass, with the meninges being particularly diffusely congested and showing occasional areas of haemorrhage and some opacity in sulcal areas. On cut surface there was also cerebral congestion and the lateral ventricles were slightly dilated with thinning of the periventricular white matter. Incidental findings included moderate mitral endocardiosis with eccentric left ventricular hypertrophy and mild tricuspid endocardiosis. The right kidney was approximately one-third larger than the left, there was a 2+ proteinuria, and 4+ blood which could have been post mortal-derived haemoglobin or red cells or contamination.

Histopathological findings

All sections of brain and cerebellum stained routinely with haematoxylin and eosin (H&E) showed marked vascular congestion especially of the meninges. Cerebral sections showed marked subarachnoidal meningitis extending down the sulci and with an exudate containing few to numerous eosinophils, as well as neutrophils, some lymphocytes, macrophages and a few plasma cells (Fig. 2). In some areas the inflammation extended into submeningeal, outer cortical regions where there was neovascularisation, occasional neuronal necrosis, gliosis, eosinophilic and mononuclear exudate and perivascular cuffing (Figs 2 & 3). Some blood vessels contained some intravascular fragments of haemolysed

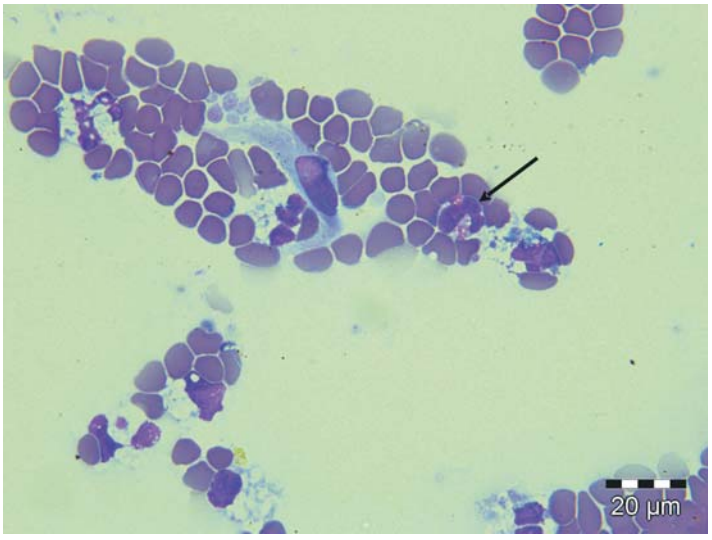

Fig.1: **Peripheral blood smear taken during the Boerboel necropsy showing neutrophils with vacuolar cytoplasm and intra-cytoplasmic irregularly-sized fragments of erythrocyte and other amorphous material; a single eosinophil** (arrow) **and a somatic spindle cell are also evident (Diff-Quik).**

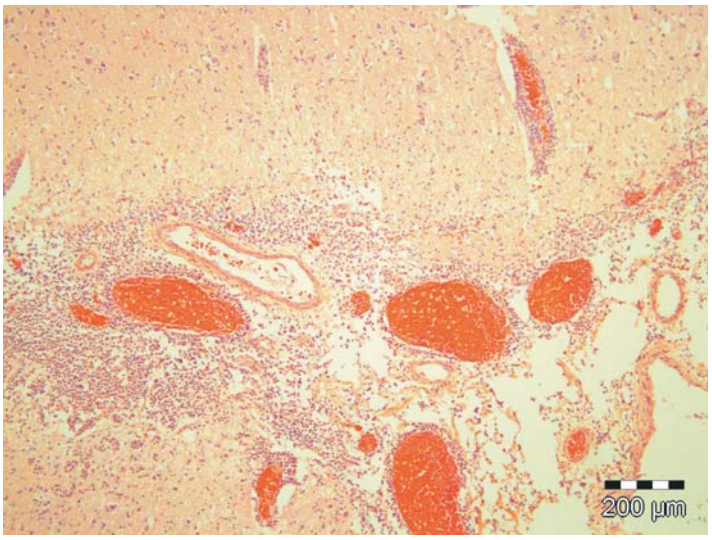

Fig. 2: **H&E stained cerebral cortex, meninges and a sulcus showing marked vascular congestion, and severe predominantly eosinophilic subpial and outer cortical meningoencephalitis in the Boerboel.**

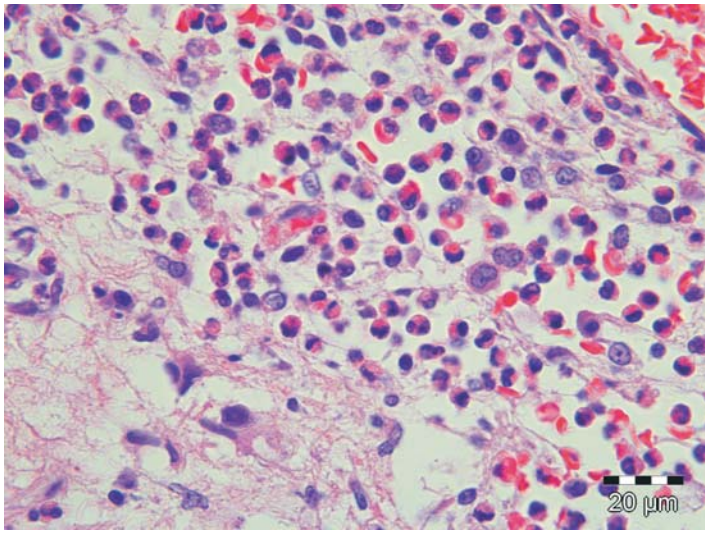

Fig. 3: **H&E stain showing numerous eosinophils in the cerebral cortical meningeal exudate (Boerboel).**

erythrocytes (microcytes)[13]. Deeper regions of the cerebrum and white matter showed multifocal perivascular cuffing with predominantly mononuclear cells (lymphocytes, plasma cells and a few macrophages) (Fig. 4).

The periventricular white matter surrounding the lateral ventricles showed multifocal locally-extensive areas of malacia with gliosis, gitter cells and phagocytosis of necrotic cellular debris and degenerated myelin. There was mild multifocal encephalitis in the hippocampus (Figs 5a & 5b).

The cerebellum and brain stem showed only mild spongiosis of some areas of white matter, especially of the peduncles, probably reflecting oedema, and meningeal congestion. Spinal cord sections also showed no significant lesions apart from 1 section showing mild multifocal peripheral white matter axon/myelin degeneration.

A kidney section was dominated by extreme congestion and there was scattered cortical and medullary single cell necrosis in tubules, with some protein and cellular debris in several lumens, signifying acute nephrosis. A section of a peripheral lymph node was congested, with sinus histiocytosis, erythrophagocytosis and some areas of red cell drainage in sinuses.

Immunohistochemical staining, using the avidin-biotin technique, of sections of cerebrum, cerebellum, spinal cord, liver, spleen, lymph node and kidney for canine distemper virus, *Toxoplasma gondii* and *Neospora caninum* against correctly-stained known positive control sections were all negative for virus or organisms.

One section of cerebrum stained with *Toxoplasma gondii*-specific immunoperoxidase stain showed a negatively-stained cyst-like structure in 1 area of the outer cortical area which appeared to contain numerous small regular-sized organisms, appearing smaller than bradyzoites of either *Toxoplasma* or *Neospora* (Fig. 6a). Using the pop-off method, transmission electron microscopy revealed this to be chromatin clumping in a neuronal nucleus with a disrupted nuclear membrane (Fig. 6b). No similar structures were found on careful examination of any other tissues with either H&E or immunohistochemical staining. Gram staining of cerebral sections also failed to show any causative organisms.

Case history 2

A 3-year-old purebred Beagle bitch had shown difficulty whelping in comparison with her 2 previous litters. With the latest litter she showed no progression of contractions, instead only shivering slightly,

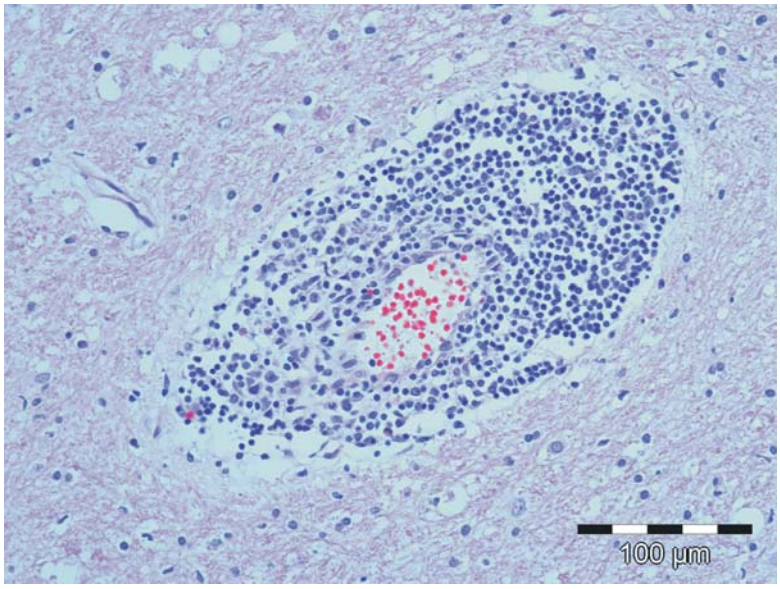

Fig. 4: **Deeper cerebrocortical white matter of the Boerboel showing multifocal perivascular mononuclear cuffing** (H&E).

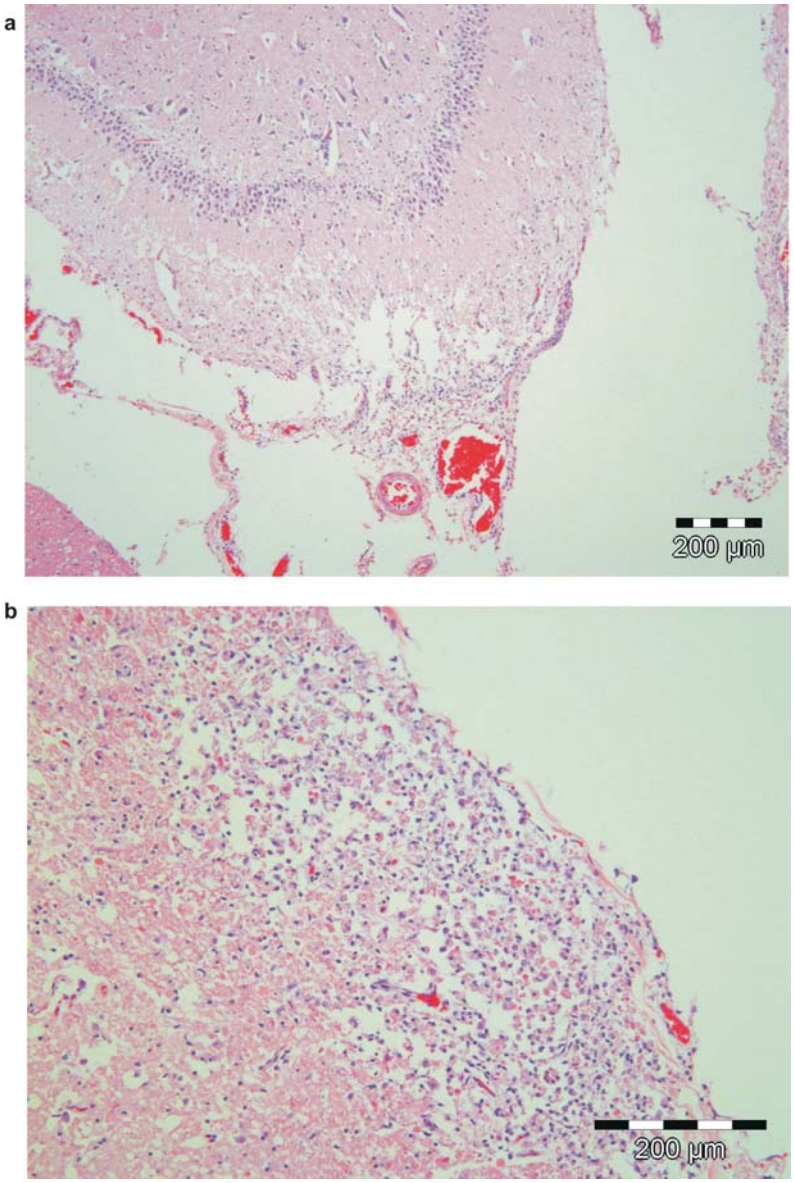

Fig. 5: a: **Boerboel's hippocampus showing multifocal malacia, local neuronal depletion, perivascular cuffing and gliosis** (H&E). b: **Periventricular white matter of the Boerboel showing malacia and gliosis** (H&E).

despite her cervix being open and the membranes of the first pup being palpable. She was given a low dose (0.4 m) of oxytocin (Fentocin, Virbac RSA, Halfway House) parenterally that still did not precipitate contractions; the vaginal discharge however increased in amount slightly and was not foul-smelling. After the 2nd larger dose (1.2 m) of oxytocin, without obvious contractions, a single pup was produced, and after another hour with no further progress she was admitted to the Onderstepoort Veterinary Academic Hospital for a Caesarian section, which proceeded uneventfully. General anaesthesia had been induced with a very low dose of Propofol (Fresenius, Kabi, Midrand) with maintenance on isoflurane gas anaesthesia *via* an endotracheal tube. All the placentae were removed, the surgical wounds were uncontaminated with uterine content and all pups were assessed to be healthy. Post-operative supportive therapy included subcutaneous amoxycillin (Clamoxyl RTU, Pfizer Animal Health, Sandton) at the dose of 20 mg/kg and intravenous crystalloid (Lactated Ringer's Solution, Adcock Ingram Critical Care, Aeroton) administered at maintenance rate over 12 hours. She was discharged 24 hours after admission with a 5-day oral course of amoxycillin-clavulanate (Synulox, Pfizer Animal Health, Sandton) at 13 mg/kg twice daily. The following 7 days post-discharge were uneventful for the dam and litter.

Nine days after the Caesarian, and 4 days after cessation of antibiotic treatment, the bitch became anorexic despite her rectal temperature being normal (reference range 36.5–39.5 C), the surgical wound was assessed to be healing well, only a small amount of pigmenturia was observed and there was no vaginal discharge. She was enticed to eat with tinned food, after which she went outside, barked at another dog, returned inside, collapsed, and subsequently started to have seizures. She was taken immediately to a private veterinarian who treated her with intravenous calcium for suspected milk fever/eclampsia but she did not respond. Diazepam also did little to stop the seizures and further doses of calcium as well as calcium borogluconate were not effective. The dog was presented to the OVAH in *status epilepticus* and with a high rectal temperature. A further amount of diazepam (10 mg in total) (Pax, Aspen Pharmacare, Sandton) and thereafter a pentobarbitone (Sodium pentobarbitone, compounded by Kyron) bolus were administered intravenously, the latter at an anti-epileptic dose of 2 mg/kg. This unexpectedly induced a deep plane of anaesthesia. A further 10 m of calcium gluconate (Calcium Borogluconate, Merial, Halfway House, South Africa) was administered as a slow bolus intravenously. Blood gas analysis after emergency treatment showed hypercalcaemia (ionised calcium of 1.65 mmol/ ; reference interval 1.15–1.32 mmol/ in humans and approximately 1.2–1.5 mmol/ in dogs), and acidosis with blood pH of 7.33 (reference interval 7.35–7.45) as measured on a Chiron Diagnostics (Rapilab 34E Chiron diagnostics, Bayer S.A.) machine. Serum creatinine was 70.3 μmol/ (Vet Test); reference interval 40–130 μmol/). Her temperature was 36.7 °C, heart rate 90/minute and respiration rate 16/minute. A blood smear was made and a stress leukogram noticed. Urine collected by cystocentesis had specific gravity of 1.040, was clear, with a pH of 5 and 2+ blood. Blood collected in heparin was tested in a hand-held machine (Ascensia Elite ts, Bayer, Isando) for glucose which was 2 mmol/ (reference interval 3.4–5.5 mmol/). Ringers lactate (Adcock Ingram Critical Care, Aeroton) was administered intravenously and spiked with 50 m of 50 % dextrose solution (creating a 2.5 % dextrose solution) and 20 mEq of potassium chloride. A 14-m , 25 % dextrose bolus was also given slowly intravenously. Her temperature increased steadily from 35.6 °C to 38.8 °C when measured at hourly intervals after admission; pulse remained at 120/minute and respiration rose from an initial 2-hourly recorded rate of 12/minute through 18/minute to 30/minute shortly before she became cyanotic and died despite being given 1 m adrenaline intravenously, 1 m intracardially, and 1 m atropine under the tongue in attempts at resuscitation.

This bitch had last been vaccinated for rabies (Rabisin, Merial South Africa, (Pty)

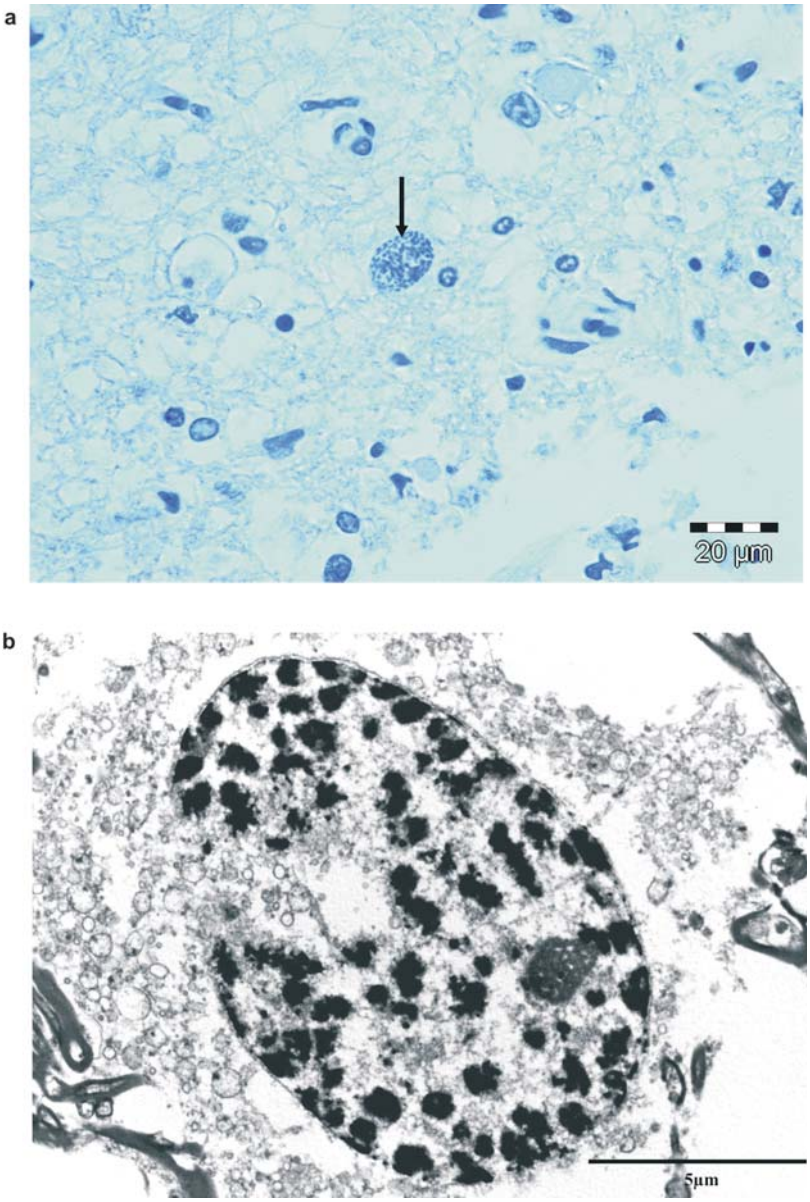

Fig. 6: a, b: **The single immunohistochemically-negative cyst-like structure** (arrow) **seen in the outer cortex in 1 of the Boerboel sections stained with *Toxoplasma gondii* antibodies.**

Ltd, Halfway House) plus a booster of a combination vaccine for distemper, hepatitis, parvovius and parainfluenza (Nobivac DHPPi, Intervet SA (Pty) Ltd, Isando, South Africa) 8 months previously. Her most recent deworming had been with a praziquantal, febantel, pyrantal embonate combination (Drontal, Bayer Animal Health Division, Isando) 64 days before whelping, just prior to mating.

Necropsy findings

The body condition of the bitch was very good. A peripheral blood smear was made but the only recorded finding was of mild red cell anisocytosis. Urine pH was 5, there was a mild proteinuria, marked glucosuria (ascribed to the dextrose administration prior to death) and 4+ blood (not recorded as to whether it was due to haemoglobin or whole red cells). The surgical wounds and uterus were unremarkable. The most striking findings were of moderate to severe generalised congestion of the carcass, severe multifocal to coalescing thymic haemorrhages (thymus not regressed); and specifically cerebral cortical congestion. There was a large (3 × 2 × 1 cm) focal caudal thoracic aortic aneurysm and a distal oesophageal mural parasitic nodule (5 × 3 × 3 cm) containing a *Spirocerca lupi* worm. Faecal floatation was not recorded.

Histopathological findings

Most significantly on H&E staining, there was severe, diffuse, especially cerebral, mononuclear leptomeningitis containing variable (few to many) numbers of intact eosinophils, extending into the sulci where the exudate was most prolific (Fig. 7), and affecting to a lesser extent the ventral meninges. The eosinophils were infiltrating singly into the outer cortical grey matter and also present around many grey and white matter blood vessels showing perivascular mononuclear cuffing (Fig. 8). The hippocampus also had perivascular cuffing multifocally of vessels but no specific peri-ventricular parenchymal lesions were noted. The cerebellar meninges were affected with only scattered mononuclear cells and eosinophils with a slight increase of the same exudate in the cerebellar sulci (Fig. 9); the cerebellar parenchyma as well as brain stem and midbrain were very little or not affected apart from intermittent mild oedema. Spinal cord was not sampled during necropsy. No light microscopically-visible evidence of any aetiological agent was found on H&E staining, and immunohistochemical staining for rabies and distemper viruses and *Neospora caninum* was negative. Acid-

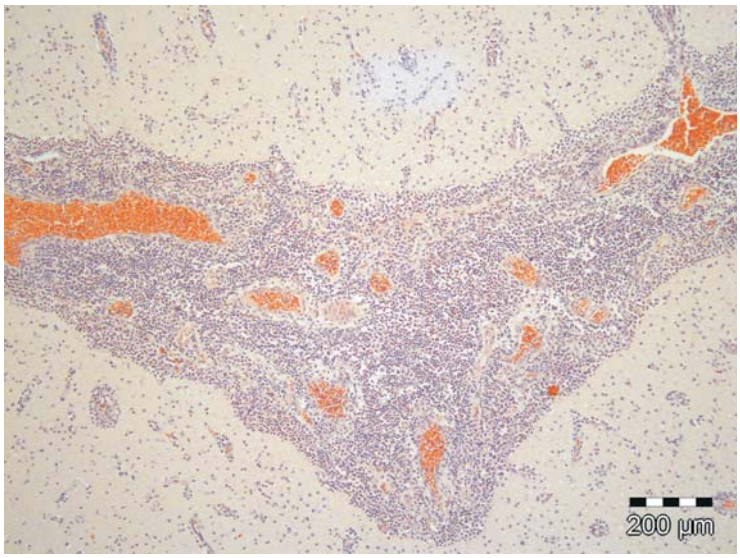

Fig. 7: **Massive meningeal exudate extending into the cerebral sulci and comprising mostly mononuclear cells and eosinophils in the Beagle bitch** (H&E).

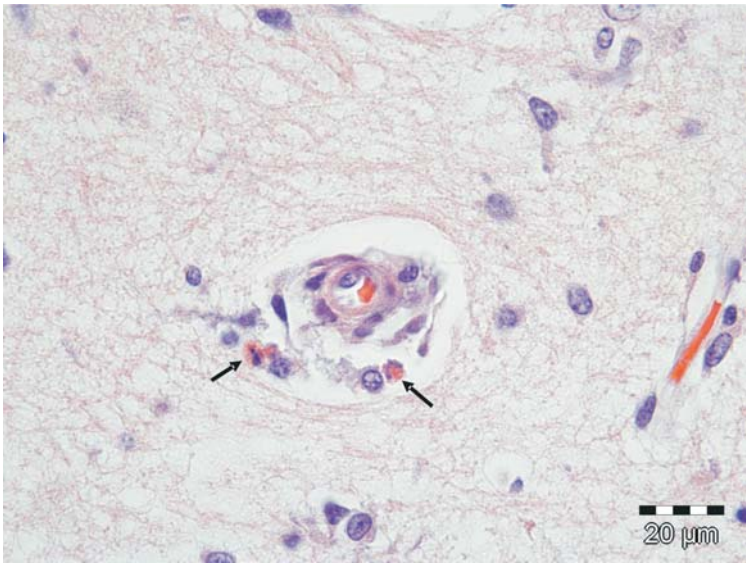

Fig 8: **Presence of eosinophils** (arrows) **in the perivascular space of many of the cerebral blood vessels of the Beagle bitch** (H&E).

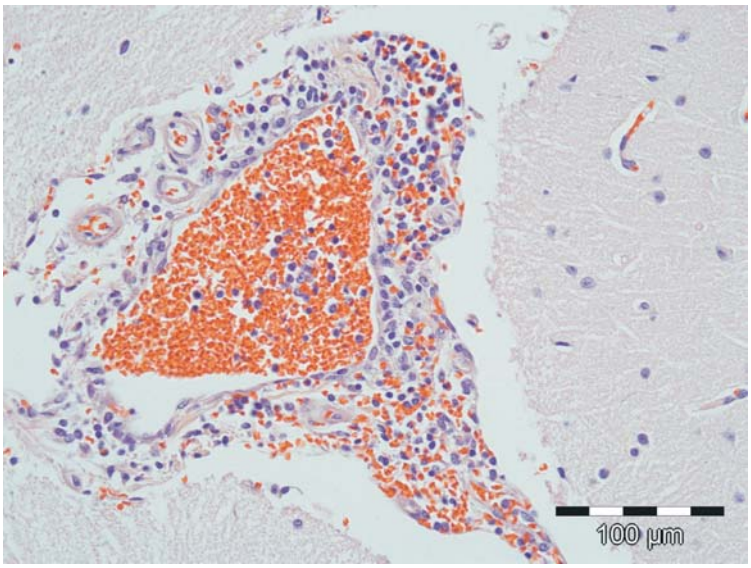

Fig. 9: **Cerebellar sulcus blood vessel of Beagle bitch showing milder perivascular mononuclear and oesinophil cuffing than in the cerebral meninges and without invasion of the molecular layer** (H&E).

fast (Ziel-Niehlsen) staining, which distinctly stains mast cell granules, showed the meningeal exudate to be devoid of mast cells.

Immunohistochemical staining for CD3 T-lymphocyte marker showed numerous T-lymphocytes in the meningeal exudate and parenchymal perivascular cuffing. CD79a staining showed scattered B lymphocytes. CD4 and CD8 specific staining could unfortunately not be performed due to the failure to store fresh brain tissue during the necropsies.

A notable finding, as seen in the Boerboel of case 1, was evidence of intravascular haemolysis, seen as small round fragments of red cells (microcytes) of varying sizes, amongst the rest of the red cells, which were also of varying sizes, in cerebral, myocardial and renal blood vessels (Figs 10 & 11). A few renal cortical tubules had a small amount of intracellular bile pigment but there were no intratubular pigment casts. The liver showed moderate intracellular bile pigment granules in hepatocytes and mild hydropic vacuolar cytoplasmic change.

The thymus showed marked multifocal to coalescing haemorrhage and was still active. The spleen was noticeably fairly bloodless in the red pulp, which had scattered megakaryocytes.

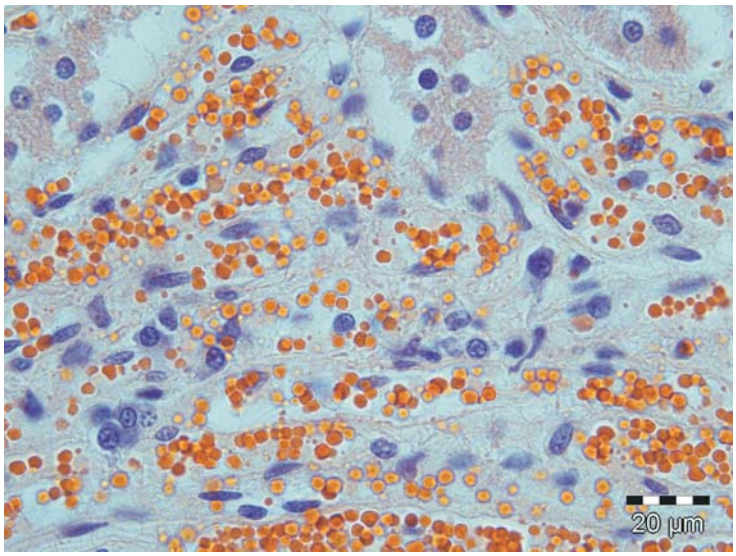

Fig 10: **Beagle bitch kidney** (H&E) **showing microcytic fragments of erythrocytes in the vasculature as well as erythrocyte anisocytosis and anisochromia.**

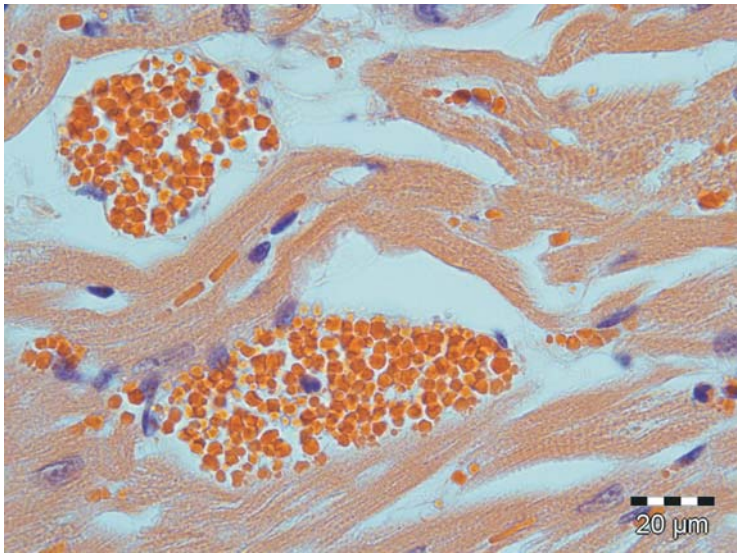

Fig. 11: **Myocardial blood vessels showing fragmented erythrocytes** (microcytes) **and anisocytosis in the Beagle bitch** (H&E)**.**

DISCUSSION

Hypereosinophilia is excessive eosinophilia and has been defined in dogs and cats as eosinophils greater than $5 \times 10^9/$. Overrepresented breeds include the Rottweiler, German Shepherd dogs, Siberian Husky, Alaskan Malamute, and Cavalier King Charles Spaniel[17].

The most common causes of hypereosinophilia are pulmonary infiltrates with eosinophils (PIE), parasitism and gastrointestinal disease[16,17]. In a study in humans the majority of cases of eosinophilia were associated with allergic processes[16]. The most common cause of eosinophilia in cats is flea allergy[16,17]. In a Swedish report investigating profound eosinophilia (exceeding the reference limit set at $2.2 \times 10^9/$) 105 dogs were found over a 2.5-year study period, and Rottweilers were over represented despite being only the 7th most common breed in Sweden[16]. Other causes of eosinophilia in dogs in this report were recorded as parasitism (although intestinal parasites have only been found in 5 % of dogs in Sweden and heartworm does not occur there); hypoadrenocorticism; acute, chronic or intermittent gastritis/enteritis; pneumonia; dermatitis mostly associated with bacterial infection and not ectoparasites; otitis externa/media; *Sarcoptes scabei* dermatitis; nasal mite (*Pneumonyssoides caninum*); and snakebite (by *Vipera berus* found in Sweden)[8].

Eosinophils live for up to 2 weeks in tissue, but only survive for 30 minutes in circulation[17]. In the brain, neurons and myelinated axons are susceptible to 'eosinophilic-induced neurotoxicity', due to neurotoxic proteins that they release, such as eosinophilic cationic protein, major basic protein, and eosinophil-derived neurotoxin[28]. Eosinophils are used in defense against parasites, are also responsible for tissue destruction as seen in the epithelial cell damage in the lungs of asthmatic patients, and they modulate inflammatory responses by producing cytokines, acting as antigen-presenting cells, and contributing to chronic inflammation and fibrosis[16]. Eosinophils generate toxic oxygen radicals *via* peroxidase activity[13] and may suppress hypersensitivity reactions by inhibiting chemical mediators liberated by mast cells during allergic and anaphylactic reactions. Local tissue production of interleukin-5, interleukin-3, and granulocyte macrophage colony-stimulating factor (GM-CSF) inhibit eosinophil apoptosis, which perpetuates an eosinophilic tissue infiltrate[24], and they also stimulate eosinophil differentiation and release into the circulation[16].

Type IV hypersensitivity is also called 'delayed type hypersensitivity' in that it takes 2 to 3 days to develop. It is mostly associated with a cell-mediated response. Predominantly T helper 2 (T_H2) lymphocytic responses are often seen in chronic inflammatory conditions with an allergic basis, such as asthma, chronic allergic inhalant or food allergic dermatitis, and chronic inflammatory bowel disease, and are associated with a humoral/antibody response[1]. As well as increasing the number of T and B lymphocytes, macro-

phages, dendritic cells and fibroblasts in the inflammatory response, T_H2 responses can also lead to increased numbers of mast cells and eosinophils, associated with immunoglobulin E and other humoral responses.

By contrast, T helper 1 (T_H1) lymphocytic, mainly cellular, chronic immunological responses often occur in response to foreign bodies, endogenous antigens such as myelin basic protein (which can occur in murine experimental allergic encephalomyelitis, an experimental model for multiple sclerosis in humans), and antigens such as the intracellular microbes *Mycobacteria* spp., *Listeria monocytogenes, Histoplasma capsulatum* and *Leishmania* spp. Some immunologists believe that the persistence of many intracellular microbes is caused by an inadequate or suboptimal T_H1 response or an inappropriately strong T_H2 response[1]. The inflammatory cells attracted to the site of T_H1 chronic inflammatory responses are T and B lymphocytes, macrophages, dendritic cells and occasionally fibroblasts. The biological characteristics of the antigens, which include the amount and structure of protein, polysaccharide and lipid, contribute to whether the resulting T-lymphocyte response will be balanced between cell-mediated (T_H1) and humoral (T_H2) responses; in many chronic inflammatory conditions there is a predominance of either 1 or the other. T helper cells comprise CD4+ T lymphocytes, whilst CD8+ T lymphocytes are suppressor or cytotoxic cells which kill target cells to which they bind, and as such are useful especially in viral infections[1].

For many eosinophilic granulomatous conditions of suspected parasitic origin in cats, dogs and horses, no specific antigen has been identified[1]. In the central nervous system, chronic inflammation, as seen with some viral infections, consists of an exudate of lymphocytes with occasional macrophages and plasma cells, which can have a perivascular distribution pattern; in exotic wildlife species and horses, in specific protozoal, parasitic or viral categories, perivascular chronic inflammatory exudates may also contain variable numbers of eosinophils[1].

The dog of case 1 in the current report was a Boerboel, which is a large working breed of dog from South Africa that resembles a Bull Mastiff and is now exported to other parts of the world[35]. This dog did not meet the criteria for hypereosinophilic syndrome (circulating eosinophils above 5×10^9/)[17], but at $3.98 \times$ 109/ , this met the requirements of the Swedish study of a 'pronounced eosinophilia' ($>2.2 \times 10^9$/)[16]. The concurrent presence of an eosinophilic infiltrate in the meninges and cerebrum, and peripheral eosinophilia, however, make this case an eosinophilic disorder. Haematology was not performed on the beagle and no record was made of eosinophils in the blood smear taken at necropsy.

The neurological syndrome in the Boerboel was assessed clinically to be a cerebellar deficit with optic tract disruption. The lack of left peripheral vision, or homonymous hemianopia, but normal direct and consensual papillary light reflexes and retinal examination, showed the localisation of the lesion to be the right lateral geniculate body of the thalamus. The lesion may have been more distal, *i.e.* involving the projections into the primary visual cortex (geniculocalcaurine tract), at the level of the calcarine sulcus (cuneus) in the visual radiation, representing the deficit on the inferior visual field quadrant, as he was tripping over obstacles but able to see people or movement in front of him. The goose-stepping gait, overshooting of the food bowl, and ataxia were consistent with a cerebellar localisation. The loss of gag reflex may have been consistent with lesions in the glossopharyngeal and vagal nerve nuclei located in the brainstem or near their common exit at the post-olivary sulcus. The neurological findings reflected the multifocal parenchymal and diffuse meningeal nature of the microscopic cerebral lesions; cerebellar signs may possibly have been due to diffuse pressure on the cerebellum by a swollen brain, since microscopic lesions of this area were minimal.

The Beagle bitch initially showed uterine inertia poorly responsive to oxytocin therapy, which necessitated a Caesarian section, and then a week later only anorexia before her sudden collapse and continuous seizures that were successfully controlled only by deep anaesthesia induced by pentobarbitone therapy. Those observing her clinical status could apparently see that this was not the typical eclampsic rigidity and tremors.

As found in 3 of the 4 previously reported cases that underwent necropsy, namely the 17-month-old Rottweiler, the 4-year-old Golden retriever and the Italian Maremma sheepdog[2,26,28], the meningeal and other significant lesions appeared to always involve only the cerebral cortex, although specific mention of the cerebellum and spinal cord in the other cases was not necessarily made or spinal cord was not always examined[2,26,28]. The cortical involvement was mainly restricted to submeningeal grey matter with some perivascular cuffing occurring in deeper regions. Only in the Boerboel were periventricular and irregular hippocampal inflammation and malacia recorded. The Beagle bitch also had encephalitis involving the hippocampus, and cerebellar meningitis was much milder than in the cortex.

No evidence of aetiology was found in any of the microscopic sections of the current cases. The outer cerebral cyst-like structure seen on light microscopy in the Boerboel was found ultrastructurally to be a neuronal nucleus undergoing classical karyorrhexis, one of the characteristics of cellular necrosis[8].

The only treatments that both Boerboel and Beagle had received prior to the start of the neurological signs were the amoxycillin-clavulanate antibiotic and combination anthelmintic; however, the Beagle had been dewormed 10 weeks prior to the onset of clinical signs. The aetiology of the Boerboel's previously diagnosed cystitis had not been pursued. The Beagle bitch did not have evidence of any bacterial infection at necropsy but did have mild active *Spirocerca lupi* infestation. *Spirocerca lupi* may uncommonly be associated with a systemic eosinophilic response during infestation but there may be a local eosinophilic granulomatous response if a worm dies in the tissues during its migratory path. Peri-aortic migration tracts and oesophageal parasitic nodules are in general surprisingly uncommonly associated with eosinophils as seen by light microscopy[33]. The possibility of a hypersensitivity response to one or more of these components cannot be excluded. It would have been of interest to attempt immunosuppressive therapy, but unfortunately the owners of the Boerboel declined further attempts at diagnosis and treatment, and the Beagle bitch was being treated as an emergency for erroneously diagnosed eclampsia and subsequently died without further diagnostic tests or treatment being performed.

Both animals interestingly had signs of recent intravascular haemolysis or erythrocyte fragmentation (microcytes) evident in histological sections of organs, and of schistocytes in the Boerboel's blood smear. This finding was not mentioned in any of the previously reported cases.

The microcytes in the H&E sections in the circulation of both dogs in this report could have been due to a fragmentation-type intravascular haemolysis and/or oxidative damage leading to Heinz body production. The Boerboel's peripheral blood smear made prior to necropsy showed numerous irregular small fragments of erythrocytes (schistocytes), indicating intravascular haemolysis, and phagocytosis of some of these shards by neutrophils; there was also eosinophilic

globular protein resorption in some of its renal tubules, possibly haemoglobin, but no intratubular pigment casts were seen. Neither dog had post mortal macroscopic or microscopic evidence of prolonged or massive extravascular haemolysis, which classically should lead to icterus, and both had only a small amount of bilirubin pigment intracellularly in various organs. Both dogs at necropsy showed generalised congestion, which may have been amplified by free haemoglobin.

Immune-mediated haemolytic anaemia due to penicillins and its congeners is rare according to a human text and is usually seen during treatment with very high doses (over 10 million units/day) for more than 2 weeks. The underlying mechanism is erythrocyte coating by penicillin to form a penicilloyl bond on their surface, with subsequent drug specific IgG antibody being directed against the complete antigen (*i.e.* the complex); clinical haemolysis therefore requires both sufficient coating of erythrocytes and high antipenicilloyl IgG titres[4]. Another 'innocent bystander' mechanism is also possible where penicillin-antibody complexes are only loosely bound to erythrocytes and activate complement. The complement may be detected on the erythrocyte surface with the complement antiglobulin test. The haemolytic reaction may apparently continue for weeks after withdrawal of the penicillin therapy, for as long as sufficient penicillin-coated erythrocytes and specific antibodies remain in circulation. The site of haemolysis in this text was not specified, but one unusual case was mentioned in a young woman who developed microangiopathic haemolysis and thrombocytopaenia in temporal relation to 3 separate courses of penicillin or ampicillin[4]. Penicillin-related haemolysis as mentioned in a veterinary text is reportedly primarily extravascular (phagocytic)[13].

Amongst many other adverse effects of β-lactam antibiotics, virtually all of this group can cause eosinophilia, either isolated or in the context of very different immunologically-mediated reactions, probably more often with meticillin, nafcillin, oxacillin, 2nd- and 3rd-generation cephalosporins, aztreonam, and imipenem[22].

Neurotoxicity has been attributed to the majority of β-lactam antibiotics and is considered to be the consequence of GABAergic inhibition, and includes clear epileptic manifestations, also documented in animals, as well as some more atypical manifestations, which in humans include drowsiness and hallucinations[22]. The affinity of various penicillins for the benzodiazepine receptor may be part of the chain of events leading to neurotoxicity. However, with penicillins and cephalosporins, integrity of the β-lactam ring is a prerequisite and epileptogenic activity is extinguished by β-lactamase. Clinical manifestations are always dose dependent, and brain tissue concentrations appear to be more relevant than CSF or blood concentrations, thus implying that impaired renal function is the major risk factor.

Drug-induced aseptic meningitis (DIAM) has been reported in humans in response to various agents, most notably non-steroidal anti-inflammatory drugs and especially ibuprofen[25], intravenous immunoglobulins, anti-CD3 monoclonal antibody (OKT3), and various antibiotics, including amoxycillin with and without clavulanic acid. Patients with a history of connective tissue disorders, *e.g.* systemic lupus erythematosus, appear to be at higher risk of DIAM[25]. Especially types 1 and 3 hypersensitivity reactions have been invoked as the cause by many investigators, supported by detection of immune complexes in the serum or CSF of some patients. However, 1 man showing fever and severe headache from 6 hours after a single 500 mg amoxycillin plus 125 mg clavulanic acid tablet, which had occurred similarly on previous occasions, showed no specific immunoglobulin E to amoxycillin or immune complexes in his serum or CSF. After cessation of treatment, the fever and headache would subside over 1 to 3 weeks[10]. In DIAM the CSF contains predominantly neutrophils as well as elevated protein, reminiscent of acute bacterial meningitis, but CSF glucose levels are usually normal[25]. One male HIV-infected patient showed DIAM induced by cotrimoxazole and had eosinophilic aseptic meningitis as well as peripheral eosinophilia. He reacted with a febrile coma within an hour after his 1st dose. The eosinophilia disappeared within a few weeks and the CSF count was normal at 7 days. He had no evidence of helminthiasis or other parasitic agents[23]. In another study 8 patients with DIAM are discussed and the time between the use of the causative drug and onset of symptoms ranged from 2 to 7 days. Clinical signs and CSF findings are mostly indistinguishable between DIAM and early stage CNS bacterial infection. One case of lymphocytic aseptic meningitis was reported in a patient with systemic lupus erythematosus, with no prior use of medications known to provoke DIAM, and no infectious agent nor chronic meningitis; the patient's symptoms resolved spontaneously[21].

Another possible explanation for the EME cases of this report is that both dogs could have had initial infections with a pathogen or different pathogens that was/were responsive to the amoxycillin but where the pathogen/s elicited the subsequent immune-mediated responses. The Boerboel had been diagnosed with cystitis 6 days before the start of clinical signs and the Beagle bitch had shown uterine inertia of undetermined origin and non-responsive to the usual recommended treatment only 9 days prior to anorexia and seizures. The trigger has been suggested to be a mild bacterial meningitis, as 1 case currently reviewed responded to chloramphenicol alone, but this has not been proven [28].

The fact that some of the previously reported cases of idiopathic canine and feline EME had a clinical response to a therapeutic trial of dexamethasone treatment suggests that the condition may have an underlying immune-mediated mechanism, with the possibility of type I and/or type IV hypersensitivity playing a role[20]. The cases reported here might suggest an immune-mediated response to penicillin since that appeared to be the only common denominator in their recent history, with a similar time lapse between start of treatment and clinical signs.

It could also be speculated that the eosinophilic meningoencephalitis of the current 2 cases may have been due to type IV hypersensitivity possibly involving exposure to 1 or more cerebral parenchymal antigens as a resulf of a break in the blood–brain barrier, and eliciting a T_H2 or perhaps a combination of T_H1 and T_H2 immune response. Experimental autoimmune encephalomyelitis (EAE) is created in mice by inoculation either with myelin basic protein or myelin oligodendrocyte glycoprotein and this is commonly used as a model for multiple sclerosis of humans for evaluation of potential therapies[14]. It has been found that the number of both CD4(+) and CD8(+) T cells and their myelin oligodendrocyte glycoprotein (MOG)-reactivity in the CNS of EAE mice were associated with increasing disease severity and that they originated in the CNS. Polymerase chain reaction analysis suggested that both CD4(+) and CD8(+) T cells produce interferon-gamma and tumour necrosis factor-alpha, while CD4(+) T cells may contribute to the induction and development of EAE by producing interleukin-17. It appears that CD4(+) T cells are involved in the early phase of EAE, whereas CD8(+) T cells have a regulatory role in the later stage[30]. EAE is characterised by a lymphocytic inflammatory infiltrate in the CNS associated with axonal degeneration, demyelination and damage.

Treatment with methylprednisolone

caused remittance of the clinical signs and the CNS infiltrate, accompanied by loss of reactivity of lymphocytes to MOG(35–55) peptide, and its withdrawal initiated relapse of the clinical signs, CNS inflammatory infiltrate and MOG reactivity of lymphocytes[5]. It was unfortunate that no fresh tissue was kept from the 2 dogs reported here for the purpose of CD4 and CD8 T-cell immunohistochemical staining.

It has recently been found that the CNS is more permissive to the development of immune responses than previously thought, and immune tolerance to neural antigens can be induced outside the CNS by elimination of autoreactive T cells in the thymus, in secondary lymphoid organs (by thymus-derived naturally occurring regulatory T cells of the CD4(+) Foxp3(+) phenotype), and by multiple mechanisms in the CNS including local activation of regulatory T cells[15].

CONCLUSION

Idiopathic EME remains a rare and enigmatic condition, which might in some cases represent a hypersensitivity-type reaction to one or more antigens. Efforts in future cases should continue to eliminate possible known aetiologies by specific tests and response to various treatments, and by careful examination of prior history, *post mortem* examinations and histopathology where appropriate, attempt to get closer to the likely cause or causes.

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REFERENCES

1. Ackermann M R 2007 Chronic inflammation and wound healing. In McGavin M D, Zachary J F (eds) *Pathologic basis of veterinary disease* (4th edn) Mosby Elsevier, China: 157, 162, 165
2. Bennett P F, Allan F J, Guilford W G, Julian A F, Johnston C G 1997 Idiopathic eosinophilic meningoencephalitis in Rottweiler dogs: 3 cases (1992–1997). *Australian Veterinary Journal* 75: 786–789
3. Bosch I, Ochmichen M 1978 Eosinophilic granulocytes in cerebrospinal fluid: analysis of 94 cerebrospinal fluid specimens and review of the literature. *Journal of Neurology* 219: 93–105
4. Cerny A, Neftel K A, Fux C, Hoigné R 2000 Penicillins. In Dukes M N G, Aronson J K (eds) *Meyler's side effects of drugs: an encyclopaedia of adverse reactions and interactions* (4th edn). Elsevier Science, Oxford: 810–820
5. Chan J, Ban E J, Chun K H, Wang S, McQualter J, Bernard C, Toh B H, Alderuccio F 2008 Methylprednisolone induces reversible clinical and pathological remission and loss of lymphocyte reactivity to myelin oligodendrocyte glycoprotein in experimental autoimmune encephalomyelitis. *Autoimmunity* 41: 405–413
6. Cook J R, Levesque D C, Nuehring L P 1985 Intracranial cuterebral myiasis causing acute lateralizing meningoencephalitis in two cats. *Journal of the American Animal Hospital Association* 21: 279–284
7. Fischman R A 1980 *Cerebrospinal fluid in diseases of the nervous system*. W B Saunders, Philadelphia
8. Ghadially F N 1988 *Ultrastructural pathology of the cell and matrix* Vol. 1 (3rd edn). Butterworths, London.
9. Jubb K V F, Kennedy P C, Palmer N 1985 *Pathology of domestic animals* Vol. 1 (3rd edn). Academic Press, Orlando
10. Kastenbauer S, Pfister H-W, Wick M 2003 No evidence of type 1 or type 3 hypersensitivity mechanism in amoxycillin/clavulanic acid induced aseptic meningitis. *Journal of Neurosurgery and Psychiatry* 74: 690–691
11. Kepa L, Oczko-Grzesik B, Stolarz W, Sobala-Szczygiel B 2005 Drug-induced aseptic meningitis in suspected central nervous system infections. *Journal of Clinical Neuroscience* 12: 562–564
12. Kuberski T 1979 Eosinophils in the cerebrospinal fluid. *Annals of Internal Medicine* 91: 70–75
13. Latimer K S, Mahaffey E A, Prasse K W (eds) 2003 Duncan & Prasse's *Veterinary laboratory medicine: clinical pathology* (4th edn). Iowa State Press, Ames
14. Leadbetter E A, Bourque C R, Devaux B, Olson C D, Sunshine G H, Hirani S, Wallner B P, Smilek D E, Happ M P 1998 Experimental autoimmune encephalomyelitis induced with a combination of myelin basic protein and myelin oligodendrocyte glycoprotein is ameliorated by administration of a single myelin basic protein peptide. *Journal of Immunology* 161: 304–512
15. Liblau R, Cassan C 2007 Immune tolerance and control of CNS autoimmunity: from animal models to MS patients. *Revue Neurologique* (*Paris*): 163: 3512–3522
16. Lilliehöök I, Gunnarsson L Z, Zakrisson G, Tvedten H 2000 Diseases associated with pronounced eosinophilia: a study of 105 dogs in Sweden. *Journal of Small Animal Practice* 41: 248–253
17. Lilliehöök I, Tvedten H 2003 Investigation of hypereosinophilia and potential treatments. *Veterinary Clinics of North America: Small Animal Practice*: 33: 1359–1378
18. McBeath A A 1980 Eosinophilic meningitis following myelography [letter]. *Journal of the American Medical Association* 243: 2396–2397
19. MacDonald J M, deLahunta A, Georgie J A 1976 Cuterebral encephalitis in a dog. *Cornell Vet* 66: 372–380
20. Maxie M G (ed.) 2007 Nervous systems. In *Jubb, Kennedy and Palmer's pathology of domestic animals* Vol. 1 (5th edn). Elsevier, China: 445
21. Mikdashi J, Kennedy S, Krumholz A 2008 Recurrent benign lymphocytic (Mollaret) meningitis in systemic lupus erythematosus. *Neurologist* 14: 43–45
22. Neftel K A, Zoppi M, Cerny, Fux C 2000 Reactions typically shared by more than one class of β-lactam antibiotics. In Dukes M N G, Aronson J K (eds) *Meyler's side effects of drugs; an encyclopaedia of adverse reactions and interactions* (4th edn). Elsevier Science, Oxford: 791–809
23. Patey O, Lacheheb A, Dellion S, Zanditenas D, Jungfer-Bouvier F, Lafaix C H 1998 A rare case of cotrimoxazole-induced eosinophilic aseptic meningitis in an HIV-infected patient. *Scandinavian Journal of Infectious Disease* 30: 530–531
24. Robinson D S, Kay A B, Wardlaw A J 2002 Eosinophils. *Clinical Allergic Immunology* 16: 43–75
25. Rodriguez S C, Olguin A M, Miralles C P, Viladrich P F 2006 Characteristics of meningitis caused by ibuprofen: report of 2 cases with recurrent episodes and review of the literature. *Medicine* (*Baltimore*) 85: 214–220
26. Salvadori C, Baroni M, Arispici M, Cantile C 2007 Magnetic resonance imaging and pathological findings in a case of canine idiopathic eosinophilic meningoencephalitis. *Journal of Small Animal Practice* 48: 466–469
27. Schultze A E, Cribb A E, Tvedten H W 1986 Eosinophilic meningoencephalitis in a cat. *Journal of the American Animal Hospital Association* 22: 623–627
28. Smith-Maxie L L, Parent J P, Rand J R, Wilcock B P, Norris A M 1989 Cerebrospinal fluid analysis and clinical outcome of eight dogs with eosinophilic meningoencephalomyelitis. *Journal of Veterinary Internal Medicine* 3: 167–174
29. Snead III O C, Kalavsky S M 1976 Cerebrospinal fluid eosinophilia. *Journal of Pediatry* 89: 83–84
30. Sonobe Y, Jin S, Wang J, Kawanokuchi J, Takeuchi H. Mizuno T, Suzumura A 2007 Chronological changes if CD4(+) and CD8(+) T cell subsets in the experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis. *Tohoku Journal of Experimental Medicine* 213: 329–339
31. Tyler D E, Lorenz M D, Blue J L, Munnell J K, Chandler F W 1980 Disseminated protothecosis with central nervous system involvement in a dog. *Journal of the American Veterinary Medical Association* 176: 987– 993
32. Vandevelde M, Spano J S 1977 Cerebrospinal fluid cytology in canine neurologic disease. *American Journal of Veterinary Research* 38: 1827–1832
33. Van der Merwe L L, Kirberger R M, Clift S, Williams M, Keller N, Naidoo V 2008 *Spirocerca lupi* infection in the dog: a review. *The Veterinary Journal* 176: 294–309
34. Vidal J E, Sztajnbok J, Seguro A C 2003 Eosinophilic meningoencephalitis due to *Toxocara canis:* case report and review of the literature. *American Journal of Tropical Medicine and Hygiene* 69: 341–343
35. Wikipedia, The Free Encyclopedia, Wikimedia Foundation, Inc, USA. Online at: http://en.wikipedia.org/wiki/Boerboel (accessed 19 February 2008)