

Papers

Serum protein electrophoresis in 147 dogs

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Reference intervals for serum protein electrophoresis (SPE) were created from a group of 75 clinically healthy dogs and compared with SPE results obtained from clinical cases presented to the University of Bristol over an eight-and-a-half-year period. A total of 147 dogs, in which SPE had been performed, had complete case records available and thus met the inclusion criteria. Signalment and final diagnoses taken from the case records and SPE results were divided into normal and abnormal based on the newly established reference intervals. Cases were grouped according to the SPE protein fraction abnormalities and diagnosis using the DAMNITV classification system. Of the 147 cases, 140 (95.2 per cent) had abnormal SPE results. The most common protein fraction abnormality was decreased albumin (59.3 per cent) followed by a polyclonal increase in γ globulins (38.6 per cent). Decreased β -1 globulins and increased β -2 globulins were documented in 36.4 and 30.0 per cent of cases, respectively. The most common DAMNITV classification associated with abnormal SPE results was infectious/inflammatory disease, which was diagnosed in 79 of 140 cases (56.4 per cent). Monoclonal gammopathies were noted in eight dogs (5.7 per cent), and underlying lymphoproliferative disease was present in all cases where a diagnosis was achieved, including multiple myeloma (four dogs), splenic plasmacytoma (one dog), hepatic plasmacytoma (one dog) and lymphoma (one dog).

SERUM protein electrophoresis (SPE) is a laboratory technique used to separate serum proteins by size and electrical charge, allowing the identification and quantification of protein fractions (Abate and others 2000, Willard and Tvedten 2004, Stockholm and Scott 2008, Cerón and others 2010). Following separation, the fractions are stained and their density is measured using a densitometer, producing a graphical trace (the electrophoretogram). The percentage area under the electrophoretogram for each protein fraction region is then calculated and multiplied by the total protein concentration to produce values for each fraction. Fractions quantified are albumin and α -1, α -2, β -1, β -2 and γ globulins (Willard and Tvedten 2004, Stockholm and Scott 2008).

Acute-phase proteins (APPs), such as α 1-lipoprotein, α 2-macroglobulin and haptoglobin, form part of the α globulin region. These are elevated in dogs with inflammation (Cerón and others 2005), neoplasia

(Vaden and others 2009), hepatic disease (Sevelius and Andersson 1995), drug treatment (Harvey and West 1987) and endocrine disease (Van den Broek 1992). The β globulins include transferrin (a negative APP), lipoproteins, complement and immunoglobulins (particularly IgM and IgA). These are increased with inflammatory, neoplastic and hepatic disease (Gavazza and Sacchini 2009, Vaden and others 2009) and decreased with protein malnutrition (O'Connell and others 2005), nephrotic syndrome (Vavricka and others 2009) and hypoadrenocorticism (Van den Broek 1992). The γ globulin fraction is composed of immunoglobulins (particularly IgG and IgE) and C-reactive protein, which are increased in established inflammation; conversely, this fraction is decreased in immunodeficiencies and some endocrine diseases (Van den Broek 1992, Willard and Tvedten 2004).

In addition to the quantification of protein fractions, the shape of the electrophoretic trace can provide diagnostic information. Monoclonal gammopathies are indicated by a tall, narrow 'spike' on the densitometer trace. These are most commonly seen in the γ region, but can be found in the β region. Monoclonal gammopathies indicate synthesis of a homogeneous immunoglobulin or immunoglobulin subunit, as the result of clonal proliferation of B lymphocytes (Giraudel and others 2002). Monoclonal gammopathies in dogs are most frequently associated with lymphoproliferative neoplasia, including multiple myeloma and lymphoma (MacEwen and Hurvitz 1977, Giraudel and others 2002), but have been associated with other diseases including infectious disease (Breitschwerdt and others 1987, Font and others 1994, De Caprariis and others 2009), inflammatory disease (Diehl and others 1992, Burkhard and others 1995), primary hyperparathyroidism (Bencheikroun and others 2009) and Waldenström's macroglobulinaemia (Gentilini and others 2005).

Previous studies have documented SPE reference intervals for normal dogs (Keay 1982, Fayos and others 2005) and found breed (Fayos and others 2005) and environmental (Keay 1982) differences, but also changes associated with a wide range of diseases. Dogs with lymphoma have been found to have increased β globulins in 53.8 per cent of cases (Gavazza and Sacchini 2009), and dogs with leishmaniosis (Ciaramella and others 1997) and ehrlichiosis (Harrus and others

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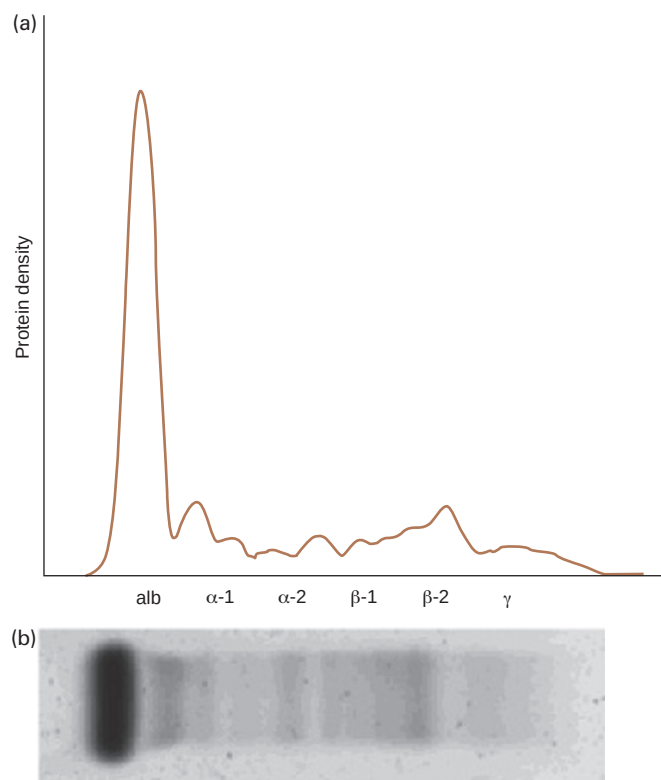


FIG 1: Example of a (a) serum protein electrophoresis densitometer trace and (b) agarose strip, illustrating the separation of proteins into different categories and subsequent quantification. alb Albumin

1996) have been commonly reported as hypoalbuminaemic and with increased β and γ globulins, while serum protein values have been published in dogs with endocrine diseases including diabetes mellitus, hypothyroidism and hypoadrenocorticism (Van den Broek 1992). Studies have also revealed that SPE results can provide prognostic information; for example, dogs with liver disease have improved survival times when hypoalbuminaemia and increased haptoglobin are present (Sevelius and Andersson 1995). The aims of the present study were to establish laboratory-specific reference intervals for SPE from a group of clinically healthy dogs and to describe normal and abnormal SPE results and associated diseases from dogs examined at a university veterinary referral hospital over an eight-and-a-half-year period.

Materials and methods

Laboratory methodology

All samples were analysed by one author (SJD) using the same methodology for each SPE. Electrophoresis was performed on 50 μ l non-haemolysed serum using agarose gels (Beckman Paragon System; Beckman-Coulter) according to the manufacturer's instructions. A GS-700 imaging densitometer (Bio-Rad) was used to analyse gels using the manufacturer's Molecular Analyst software. The product of percentage volume of each fraction and total protein concentration, measured using the biuret method (Konelab; Inverness Medical), was calculated to determine individual fraction concentration in grams per litre (Fig 1).

Calculation of SPE reference intervals

The reference intervals for each fraction were created following analysis of serum from 75 clinically normal dogs (the reference group). The serum was obtained with owner consent from residual samples taken as part of the Pet Travel Scheme (VLA 2009). At the time of sampling, each dog was examined by a veterinary surgeon and confirmed to be clinically healthy. The reference intervals were established using 95 per cent of the healthy population and excluding outliers (Geffré and others 2009).

SPE results from clinical cases

Clinical case records of dogs examined at the University of Bristol from April 2001 to October 2009 were searched and all cases in which

TABLE 1: Reference intervals for canine serum protein electrophoresis

Region	Albumin	α -1	α -2	β -1	β -2	γ
Lower reference interval (g/l)	27.20	3.38	2.37	2.90	2.17	2.26
Upper reference interval (g/l)	44.90	9.09	9.58	10.70	5.81	10.70

SPE was performed as part of the diagnostic investigation were selected. Cases meeting the inclusion criteria of complete case records and SPE results formed the 'clinical group'. Information on signalment and final diagnoses was obtained for each case and SPE results were compared with the established reference interval. Cases within the clinical group were then categorised as 'normal' or 'abnormal' and further divided according to the abnormal protein fraction. Cases with elevated γ globulins were classified as polyclonal if a wide-based γ globulin fraction was recorded on the SPE trace. Monoclonal gammopathies were classed as such if the electrophoretic trace showed a narrow, spike-like peak with a base similar in width to the albumin band or a peak height at least four times the peak width (Giraudel and others 2002, Vaden and others 2009).

Cases were also categorised by aetiology according to the DAMNITV classification as degenerative, anomalous, metabolic, neoplastic, inflammatory/infectious, idiopathic, toxic/traumatic or vascular (Garosi 2004, Rizzo and others 2007, Taylor and others 2010). A miscellaneous group included cases that did not fit these categories and cases with no diagnosis were also recorded. Where more than one diagnosis was recorded, cases were highlighted and included in all relevant DAMNITV categories, while contributing only once to the total number of cases with each protein fraction abnormality.

Statistical analysis

Data were tested for normality using the D'Agostino and Pearson omnibus normality tests. The chi-squared test, two-tailed Student's *t* test or Mann-Whitney U test were used to assess differences in signalment between the reference group and the clinical group depending on whether categorical, normally distributed or non-normally distributed data were being analysed, respectively. During creation of the reference intervals, the Grubbs' outlier test was used to determine outliers that were then removed. Statistical analysis was performed using a statistical package (GraphPad Prism v5 for Windows; GraphPad Software) and significance was set at $P < 0.05$.

Results

Calculation of SPE reference intervals

The reference intervals are shown in Table 1. SPE was performed on 75 clinically normal dogs (reference group) including 16 non-pedigree crossbreed dogs and 59 pedigree dogs; the latter group was comprised of nine labrador retrievers, six German shepherd dogs, five Jack Russell terriers, four border collies, four cocker spaniels, three boxers, three golden retrievers, two each of border terriers, dachshunds, springer spaniels, flat-coated retrievers, toy poodles and West Highland white terriers, and one each of bearded collie, chihuahua, dobermann, English setter, Italian greyhound, Lhasa apso, old English sheepdog, Patterdale terrier, standard poodle, Staffordshire bull terrier, whippet, Yorkshire terrier and Maltese terrier. The median age of dogs in the reference group was 2.5 years (range four months to 13 years). The group featured 44 (58.7 per cent) male dogs (31 entire and 13 neutered) and 31 (41.3 per cent) female dogs (23 entire and eight neutered). Following analysis, seven samples were classed as outliers and excluded.

SPE results from clinical cases

Of 177 cases with SPE performed, only 147 met the inclusion criteria (clinical group). Thirty cases were excluded because of missing or incomplete clinical records. The clinical group consisted of 15 non-pedigree crossbreed dogs and 132 pedigree dogs that included 12 each of German shepherd dogs, springer spaniels and golden retrievers, eight each of labrador retrievers and cocker spaniels, seven border collies, six boxers, five each of Jack Russell terriers and bullmastiffs, four

TABLE 2: Serum protein electrophoresis (SPE) protein fraction abnormalities and DAMNITV diagnoses in 140 dogs*

Abnormality (number of cases[%])	ND (29)	Degenerative (1)	Anomalous (5)	Metabolic (2)	Neoplastic (21)	Infectious/ inflammatory (79)	Idiopathic (3)	Toxic (2)	Vascular (3)	Miscellaneous (9)
Decreased albumin (83 [59.3])	16		PSS (1), Portal vein hypoplasia (1), Renal dysplasia (1)		<i>Multiple myeloma</i> (3), <i>Hepatic plasmacytoma</i> (1), Splenic plasmacytoma (1), Pancreatic adenocarcinoma (1), Hepatic adenocarcinoma (2), Gastric carcinoma (1), Acute lymphoblastic leukaemia (1), Chronic granulocytic leukaemia (1), <i>Lymphoma</i> (1)	Chronic hepatitis (6), Cholangiohepatitis (3), Chronic hepatitis/cirrhosis (1), Lobular dissecting hepatitis (1), Otitis externa/digital furunculosis (1), Otitis externa/atopy (1), Granulomatous dermatitis/panniculitis (1), Pancreatitis (1), Glomerulonephritis/PTE (1) , Glomerulonephritis (1), SLE (1), IMPA (type I) (3), IMPA (<i>Borrelia</i> positive, type II) (1), SRM and IMPA (1), SRM (1), IMT (1), IMN (2), Lymphadenitis (cause unknown) (2), Stomatitis (1), Myositis (2), Diskospondylitis (4), Septic arthritis (1), Abscess and sepsis (1), Sublumbar abscess (1), Aspiration pneumonia (1), Bacterial splenitis (1), Ehrlichiosis and leishmaniosis (1), Leishmaniosis (1), Fungal infection (<i>Exophiala</i> species) (1), Gastric ulceration/gastritis (1), IBD (2), Glaucoma (1)	Idiopathic epilepsy (1)	Toxic hepatopathy (tetracycline associated) (1)	Glomerulonephritis/PTE (1) , Glomerulonephropathy (1), Lymphangiectasia (1)	
Increased α-1 (27 [19.3])	4	Type II intervertebral disc herniation (1)	Renal dysplasia (1)	CKD/UTI (1)	Parathyroid adenoma (1), Mammary carcinoma (1), Lymphoma (1)	Cholangiohepatitis (1), Demodecosis (1), Atopy (1), SRM (2), Eosinophilic bronchopneumopathy (1), IMN (1), Pancreatitis (1), Chronic bronchitis (1), Diskospondylitis (1), CKD/UTI (1) , IBD/lymphangiectasia (1) , Glaucoma (1)	Idiopathic epilepsy (1)	Toxic hepatopathy (tetracycline associated) (1)		IBD/lymphangiectasia (1) , Glomerulonephropathy (1), Pure red cell aplasia (1), Oesophageal dysmotility (1)
Decreased α-1 (8 [5.7])	1				<i>Splenic plasmacytoma</i> (1), Hepatic carcinoma (1)	Chronic hepatitis (1), Lobular dissecting hepatitis (1), Cholangiohepatitis (1), Pancreatitis (1), IBD (1)				
Increased α-2 (18 [12.9])	4			Diabetes mellitus (1)	Hepatic carcinoma (1), Lymphoma (1)	Cholangiohepatitis (1), Pancreatitis (1), Lymphadenitis (cause unknown) (2), IMHA (1), SRM and IMPA (1), Granulomatous dermatitis/panniculitis (1), Aspiration pneumonia (1), Bacterial glossitis (1), Fungal infection (<i>Exophiala</i> species) (1)				Gastric foreign body (1)
Decreased α-2 (19 [13.6])	2		PSS (1)		<i>Multiple myeloma</i> (1), <i>Splenic plasmacytoma</i> (1), Hepatic carcinoma (1), <i>Lymphoma</i> (1)	Chronic hepatitis (4), Chronic hepatitis/cirrhosis (1), Pancreatitis (1), Otitis externa and atopy (1), IBD/lymphangiectasia (1) , IMPA (<i>Borrelia</i> positive, type II) (1), Chronic bronchitis (1)				IBD/lymphangiectasia (1) , Megaesophagus (cause unknown) (1), Glomerulonephropathy (1)
Increased β-1 (11 [7.9])					<i>Multiple myeloma</i> (2), Acute lymphoblastic leukaemia (1), Pancreatic adenocarcinoma (1), Parathyroid adenoma/sertoli cell tumour (1)	Otitis externa/interdigital furunculosis (1), Otitis externa/atopy (1), Lymphadenitis (cause unknown) (1), Chronic stomatitis (1), Myositis (1), <i>Angiostrongylus vasorum</i> infection (1)				

TABLE 2: Continued

Abnormality (number of cases[%])	ND (29)	Degenerative (1)	Anomalous (5)	Metabolic (2)	Neoplastic (21)	Infectious/ inflammatory (79)	Idiopathic (3)	Toxic (2)	Vascular (3)	Miscellaneous (9)
Increased β-2 (43 [30])	5				Acute lymphoblastic leukaemia (1), Pancreatic adenocarcinoma (1), <i>Hepatic plasmacytoma</i> (1), <i>Lymphoma</i> (1), Parathyroid adenoma and sertoli cell tumour (1)	Chronic hepatitis (4), Pancreatitis (1), Atopy (1), Otitis externa and interdigital furunculosis (1), Otitis externa and atopy (1), Lymphadenitis (cause unknown) (1), SLE (1), IMPA/ <i>Borrelia</i> positive (type II) (1), Masticatory myositis (2), Myositis (1), Diskospondylitis (3), Septic arthritis (2), Bacterial glossitis (1), Fungal infection (<i>Exophiala</i> species) (1), Bacterial splenitis (1), <i>Angiostrongylus vasorum</i> infection/ pulmonary hypertension (2), <i>Angiostrongylus vasorum</i> infection (1), Demodicosis (1), Ehrlichia and leishmania (1), Leishmania (1), Chronic stomatitis (1), Chronic bronchitis (1), Gastric ulceration/gastritis (1)	Idiopathic epilepsy (1)	Toxic hepatopathy (unknown cause) (1)	<i>Angiostrongylus vasorum</i> infection/ pulmonary hyper- tension (2)	
Decreased β-2 (8 [5.7])	1				<i>Multiple myeloma</i> (1), Hepatic carcinoma (1)	Aplastic anaemia (1), IBD (1), Bacterial meningitis (1), IMPA (1)				Lymphangiectasia (1)
Increased γ (54 [38.6])	8		Portal vein hypoplasia (1), Haemorrhagic branchial cysts (1)		<i>Multiple myeloma</i> (2), <i>Splenic plasmacytoma</i> (1), Lymphoma (2), Acute lymphoblastic leukaemia (1), Gastric carcinoma (1), Parathyroid adenoma and sertoli cell tumour (1)	Chronic hepatitis (7), Chronic hepatitis/cirrhosis (1), Cholangiohepatitis (1), Otitis externa/ interdigital furunculosis (1), Otitis externa/atopy (1), Masticatory myositis (2), Lymphadenitis (cause unknown) (1), SLE (1), IMPA (type I) (2), IMN (1), IMPA/ <i>Borrelia</i> positive (type II) (1), Aplastic anaemia (1), Gastric ulceration/gastritis (1), Megaesophagus/IMPA (1), Superficial pyoderma/ atopy (1), Diskospondylitis (1), Sublumbar abscess (1), Septic arthritis (1), Bacterial splenitis (1), Ehrlichiosis/leishmaniosis (1), Leishmaniosis (1), Chronic stomatitis (1), Chronic bronchitis (1), Myositis (1)	Idiopathic epilepsy (1)	Toxic hepatopathy (unknown cause) (1)	Megaesophagus/ IMPA (1) , Pure red cell aplasia (1), Oesophageal dysmotility (1)	
Decreased γ (2 [1.4])	1				<i>Multiple myeloma</i> (1)					

* Total number of cases in each category is shown in brackets; bold type indicates cases with more than one DAMNITV category and therefore featured in all applicable categories but counting only once towards the total cases with the protein fraction abnormality; italic type indicates cases with monoclonal gammopathies
 CKD Chronic kidney disease, IBD Inflammatory bowel disease, IMHA Immune-mediated haemolytic anaemia, IMN Immune-mediated neutropaenia, IMPA Immune-mediated polyarthritis, IMT Immune-mediated thrombocytopenia, ND Diagnosis not reached, PSS Portosystemic shunt, PTE Pulmonary thromboembolism, SLE Systemic lupus erythematosus, SRM Steroid-responsive meningitis, UTI Urinary tract infection

West Highland white terriers, three each of German shorthair pointers, old English sheepdogs and Scottish terriers, two each of Bichon Frise, cavalier King Charles spaniel, dobermann, fox terrier, great dane, Irish setter, lurcher, rottweiler, weimaraner and Yorkshire terrier, and one each of Afghan hound, bearded collie, Bernese mountain dog, blood hound, border terrier, briard, Chien de Bordeaux, dalmatian, English bull terrier, flat-coated retriever, giant Schnauzer, Italian spinone, Leonberger, Lhasa apso, Maltese terrier, miniature schnauzer, miniature dachshund, rough collie, Staffordshire bull terrier, Siberian husky, Shetland sheepdog, standard poodle, Tibetan terrier and whippet. The median age of the clinical group was six years (range six months to 13 years). This group consisted of 79 (53.7 per cent) male

dogs (49 neutered, 30 entire) and 68 (46.3 per cent) female dogs (45 neutered, 23 entire).

Normal SPE results

Seven dogs in the clinical group (4.8 per cent) had normal SPE results, including five dogs with no diagnosis, one with cutaneous plasmacytoma and one with aspiration pneumonia of unknown cause.

Abnormal SPE results

The remaining 140 dogs (95.2 per cent) had abnormal SPE results (Table 2). The most frequent reason for SPE analysis in these animals was an increased serum globulin level on routine biochemistry. The

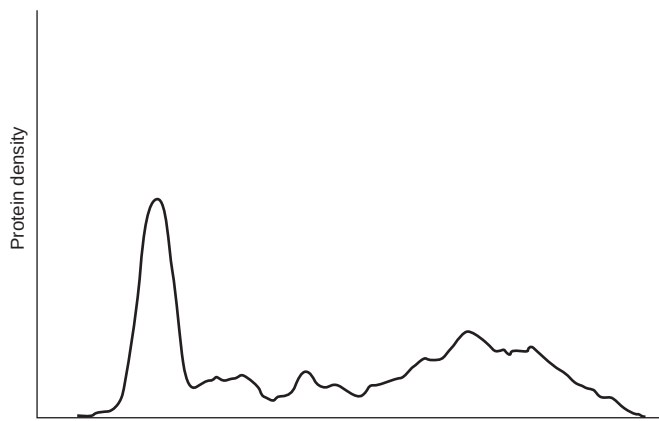


FIG 2: Serum protein electrophoresis densitometer trace from a dog diagnosed with diskospondylitis, illustrating decreased albumin and increased γ globulins, the most frequently documented combination of protein fraction abnormalities in the present study

most common protein fraction abnormality was decreased albumin (83 cases [59.3 per cent]), followed by increased γ globulins (54 cases [38.6 per cent]), decreased β -1 globulins (51 cases [36.4 per cent]) and increased β -2 globulins (43 cases [30 per cent]).

Infectious/inflammatory disease was the most frequent DAMNITV classification diagnosed in 79 cases (56.4 per cent), followed by neoplasia in 21 cases (15.0 per cent). A diagnosis was not reached in 29 cases (20.7 per cent). Among the dogs with infectious/inflammatory disease, hepatic disease (including chronic hepatitis, cholangiohepatitis and lobular dissecting hepatitis) was the most common diagnosis, accounting for 15 of the 79 cases (19.0 per cent). Further diagnoses within DAMNITV groups are listed in Table 2.

The majority of dogs with abnormal SPE results had more than one abnormal protein fraction (102 cases [72.9 per cent]). Decreased albumin and increased globulins were present in 37 cases (illustrated in Fig 2), of which the majority (24 of 37 cases) had inflammatory/infectious disease. In 31 cases, decreased albumin was accompanied by decreased β -1 globulins with diagnoses distributed between DAMNITV groups.

Monoclonal gammopathies

Of the 140 dogs with abnormal SPE results, eight (5.7 per cent) demonstrated monoclonal gammopathies, seven of which had lymphoproliferative disease (Table 3). In one case, no cause for the monoclonal gammopathy was identified; the dog, which was the oldest in the current study (13 years old), presented for investigation of diarrhoea, but following the identification of monoclonal gammopathy, further investigation was declined by the owner and the dog was lost to follow-up. Monoclonal spikes were seen in the γ globulin region for four dogs, the β region for three dogs and the α -2 region for one dog (Fig 3). In one dog with multiple myeloma, an agar gel diffusion test later identified the monoclonal spike in the β -1 region as IgM.

Signalment comparisons between the reference and clinical groups

The reference group was significantly younger than the clinical group ($P < 0.0001$) and contained significantly fewer pedigree dogs than the clinical group ($P = 0.039$). There was no significant difference in sex distribution between the groups.

Discussion

Indications for SPE in human beings include hyperproteinaemia and suspicion of plasma cell neoplasia (Attalman and Levinson 2000, O'Connell and others 2005), as well as the investigation of hepatic and gastrointestinal disease (Vavricka and others 2009). In dogs, previous reports have described SPE patterns seen in hepatic disease (Sevelius and Andersson 1995), neoplasia (Gavazza and Sacchini 2009), endocrine disease (Vaden and others 2009) and infectious disease (Harris and others 1996, Ciaramella and others 1997), as well as conditions resulting in monoclonal gammopathies on SPE (MacEwen

TABLE 3: Location of monoclonal 'spike' and diagnosis in dogs with monoclonal gammopathies

Location of monoclonal spike	Diagnosis (number of dogs)
α -2	Multiple myeloma (1)
β -1	Multiple myeloma (1)
β -2	Splenic plasmacytoma (1)
	Lymphoma (stage V) (1)
γ	ND (1)
	Multiple myeloma (2)
	Hepatic plasmacytoma (1)
ND No diagnosis reached	

and Hurvitz 1977, Giraudel and others 2002). Feline SPE findings associated with various disease processes have recently been reported (Taylor and others 2010), along with SPE findings associated with specific infectious diseases including feline infectious peritonitis (Sparkes and others 1991), feline leukaemia virus and feline immunodeficiency virus (Miró and others 2007). However, to the authors' knowledge, this is the first study to examine and report SPE findings in a large group of dogs with a variety of disease aetiologies.

The initial aim of the present study was to create reference intervals for SPE in dogs. Ideally, each laboratory should create its own reference intervals as methodology varies between laboratories and, therefore, reference values established in different laboratories cannot be used interchangeably (Keay 1982). In the current study, all SPE analyses were performed by the same person using the same methodology, thereby minimising interassay variability. The ideal reference group used to create reference intervals should be representative of the study population and factors potentially affecting the measured parameters should be controlled (Geffré and others 2009). In the current study, the dogs in the reference group were examined at the time of blood sampling and reported to be healthy. However, factors such as diet, stress, environment and the presence of occult disease were not controlled, and thus may have influenced the SPE results (Keay 1982). The dogs in the reference group were significantly younger than those in the clinical group, although no significant age-related effect could be found when comparing older and younger dogs within the control group. The distribution of pure breed dogs was similar between the two groups, although the reference group included proportionally more crossbred dogs. Variation in serum proteins between dog breeds may occur (Keay 1982); for example, greyhounds have significantly lower α and β globulins than non-greyhound breeds (Fayos and others 2005). Despite the reference group not being as representative of the study population as intended, the reference intervals created from this group were still used in the current study because limitations on the blood sampling of healthy animals in the UK meant a more suitable reference group could not be identified.

The second aim of the study was to describe the SPE abnormalities identified in a group of clinically ill dogs referred to a university veterinary hospital. In the majority of cases (95.2 per cent), the SPE results were abnormal, mainly because clinicians were performing SPE on cases in which SPE abnormalities were likely to be present, for example, cases with hyperproteinaemia or cases with suspected diseases associated with SPE abnormalities such as multiple myeloma. The diagnoses were grouped according to the DAMNITV classification scheme as has previously been reported (Garosi 2004) and was recently used to categorise diagnoses associated with feline SPE abnormalities (Taylor and others 2010). This method of classification is arbitrary and subject to individual interpretation, but allows grouping of large amounts of data and is intended to provide the reader with a clinically relevant reference for the identification of disease aetiologies associated with SPE protein fraction abnormalities.

In the present study, the most frequent DAMNITV diagnosis was infectious/inflammatory disease, including a wide variety of immune-mediated diseases, bacterial diseases and protozoal diseases. Hepatic disease was identified most frequently in this group, most commonly chronic hepatitis (all of which were diagnosed by surgical or Tru-cut biopsy). The results of this study are consistent with those of a previous study that showed that dogs with inflammatory hepatopathies

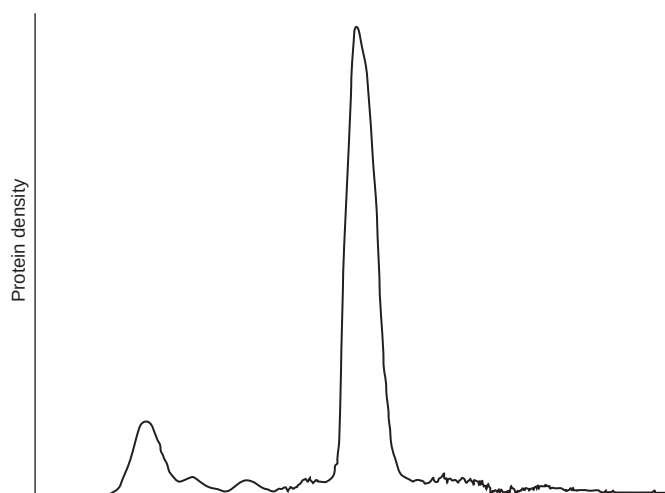


FIG 3: Serum protein electrophoresis densitometer trace illustrating monoclonal gammopathy with the monoclonal 'spike' in the α -2 region and also exhibiting profound background suppression

demonstrated decreased albumin and increased α and γ globulins (Sevelius and Andersson 1995).

The most common pattern of SPE abnormalities was decreased albumin and a polyclonal increase in γ globulins. This non-specific electrophoretic pattern suggests chronic antigenic stimulation and does not allow differentiation between various neoplastic and inflammatory diseases, illustrating the limitations of SPE as a diagnostic test (Stockholm and Scott 2008). Since albumin is a negative APP, hepatic production decreases to maintain oncotic pressure and albumin concentration can be underestimated by SPE; these factors probably contributed to the large number of dogs with reduced albumin seen in the present study (Willard and Tvedten 2004).

A large number of dogs in the present study had β globulin abnormalities, most commonly decreased β -1 globulins (36 per cent). One of the β -1 globulins is transferrin, a negative APP that is found to be reduced in the acute-phase response seen in inflammatory disease. This may explain the number of dogs in the inflammatory group with this SPE abnormality (Stockholm and Scott 2008). Five of the dogs with monoclonal gammopathy also had reduced β -1 globulins, together with reduced albumin, which may represent the 'background suppression' of other protein fractions seen in hyperglobulinaemic states (Willard and Tvedten 2004, Stockholm and Scott 2008).

In addition, β -2 globulins were increased in 30 per cent of the cases. As the main proteins migrating to the β -2 region include complement and immunoglobulins, it is not surprising that elevations in this fraction are seen with inflammatory and neoplastic disease.

In the present study, monoclonal gammopathies were found infrequently (5.7 per cent cases), consistent with previous reports (MacEwen and Hurvitz 1977, Giraudel and others 2002). All but the one case in which no diagnosis was made were associated with lymphoproliferative disease. Previous reports have also associated monoclonal gammopathies in dogs with infectious diseases, including leishmaniosis (Font and others 1994), ehrlichiosis (Breitschwerdt and others 1987) and heartworm disease (De Caprariis and others 2009). Although these infections are uncommon in the UK, two dogs in the current study were diagnosed with *Ehrlichia* and/or *Leishmania* infection, both of which exhibited patterns of hypoalbuminaemia and polyclonal rather than monoclonal gammopathy. Ideally, immunoelectrophoresis is performed on all cases with a sharp 'spike' in the electrophoretic trace, to exclude other causes such as a restricted or oligoclonal gammopathy (Stockholm and Scott 2008). In the present study, only one case with a monoclonal gammopathy was confirmed in such a way. The finding of a monoclonal spike in the α -2 region in one dog with multiple myeloma is unusual, but this may be explained by the diversity in immunoglobulin charge characteristics that in some cases can result in a shorter migration from the anode (Attalman and Levinson 2000). Based on the findings of the present study, the observation of a monoclonal spike on the SPE trace should prompt

further investigation for underlying lymphoproliferative neoplasia, as well as excluding other previously reported causes of monoclonal gammopathies such as infectious and inflammatory disease (MacEwen and Hurvitz 1977, Giraudel and others 2002).

One limitation of the present study is that the exact nature of the proteins present in each area was not determined. Abate and others (2000) used the same methodology as the present study to form SPE traces in both normal and diseased dogs; they later identified the proteins present within the discrete regions. The exact proteins present in each area of the SPE trace in the present study are speculative, but assumed to be similar to those found by Abate and others (2000). SPE is a common test, especially since immunoelectrophoresis is expensive and not readily available commercially. It was therefore deemed by the authors that the changes in SPE pattern, rather than individual protein changes, represent important diagnostic information for attending clinicians. Determination of individual protein changes represents a possible area for future investigation.

In conclusion, the practical uses, as well as limitations, of SPE in the diagnostic evaluation of canine cases are illustrated by the findings of the present study. The most common protein fraction abnormalities identified were decreased albumin and a polyclonal increase in γ globulins. This pattern was most commonly associated with inflammatory/infectious or neoplastic disease, but was not specific to any condition within these groups. Monoclonal gammopathies were uncommon but usually associated with lymphoproliferative disorders.

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