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Serum proteins and their diagnostic utility in veterinary medicine: a review

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ABSTRACT: The evaluation of serum proteins and their electrophoretic pattern is a well-established laboratory method in the diagnosis of many diseases in humans, which has replaced the biochemical determination of the concentrations of albumin and the ratio of albumin to globulins. The measurement of serum proteins may be an important diagnostic tool for the detection, diagnosis, and monitoring of various diseases and pathological processes. The results of serum protein electrophoresis can be one of the most useful diagnostic aids in a wide spectrum of diseases, including infectious and inflammatory diseases, renal or gastrointestinal disorders, immunodeficiency states, as well as paraproteinaemias caused by lymphoid or plasma cell neoplasia. Although many studies have been carried out to determine the usefulness of the determination of serum proteins and their electrophoretic pattern in various disease conditions and disorders also in animals, serum protein evaluation is still a relatively little-used diagnostic tool in veterinary medicine. In this article, methods of serum protein determination, their diagnostic utility in animal care practice and their different patterns in dysproteinaemias and paraproteinaemias are reviewed.

Keywords: animals; serum proteins; electrophoresis; diagnosis; dysproteinaemia; paraproteinaemia

Contents

1. Serum proteins – introduction

Proteins, macromolecules built from one or more unbranched chains of amino acids linked by peptide bonds, are the major components of the blood serum or plasma and have many different functions. Most serum proteins are biochemically not pure, since they are combined with other substances, for example, they can be conjugated to carbohydrate and be present in the circulation as glycoproteins (Stockham and Scott 2002). The chemical properties of the amino acids determine the biological activity of the protein (Tymchak 2010). Proteins are involved in almost all of the reactions occur-

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ring in the organism, including the maintenance of colloid osmotic structure, catalysis of biochemical reactions and buffering acid-base balance. Some of them act as carriers of lipids, hormones, vitamins and minerals in the circulatory system, and are involved in the regulation of cellular activity and the immune system (Anderson and Anderson 2002). Other blood proteins play important roles as enzymes, complement components or protease inhibitors. Certain blood proteins are essential for haemostasis and have important functions in platelet adhesion and aggregation, as well as coagulation (Meyer and Harvey 2004).

Almost all serum proteins are produced and secreted by hepatocytes. The major exceptions are the immunoglobulins that are produced by the immune system consisting of the reticuloendothelial tissues, lymphoid and plasma cells (Eckersall 2008). Further studies have shown that non-hepatic tissues, including the intestine, lung, adipose tissue and mammary gland, also have the capability of synthesising some serum proteins for specific functions (Friedrichs et al. 1995; Vreugdenhil et al. 1999).

Wrotnowski (1998) estimated that up to 10 000 proteins may commonly be found in blood serum. The more heavily represented serum proteins include albumin, immunoglobulins, haptoglobin, transferrin and lipoproteins, and they are found in concentrations that can be measured in g/ml (Burtis and Ashwood 2001). In addition to these major constituents, blood serum also contains many other proteins that are secreted by cells and tissues at very low concentrations (measured in ng/ml or pg/ml) and in veterinary clinical biochemistry are relatively understudied (Kennedy 2001; Schrader and Schulz-Knappe 2001). Protein concentrations in serum are tightly controlled to balance their physiological functions in areas of immunity, coagulation, small molecule transport and inflammation. Any dysfunction or loss of balance in the concentrations of serum proteins can cause or result from disease processes (Pieper et al. 2003).

2. Analysis of serum proteins

2.1. Total protein

The determination of the concentrations of serum proteins and the evaluation of their changes during the disease process is fundamental for their use as valid biomarkers (Okutucu et al. 2007). The quantification of the total serum protein concentration represents a basic step in general biochemistry and routine clinical laboratory practice. Several methods have been developed for the determination of total protein in serum or plasma, which are based on different analytical methods (Zaia et al. 1998). Chemical, as well as physical methods are available to measure total protein in biological fluids. In clinical biochemistry, chemical methodologies are more frequently used because of the possibility to adapt these techniques to automated analysers (Eckersall 2008).

The biuret assay is one of the oldest and most widely used assays for the determination of total protein concentrations. It is based on a colorimetric (spectrophotometric) technique, in which protein forms a violet-coloured polypeptide chelated with cupric ion, in strong alkaline solution (Gornall et al. 1949; Boyer 2000). However, this method is not sensitive enough to measure lower protein concentrations found, for example, in cerebrospinal fluid (Switzer and Garrity 1999). Despite this disadvantage, the biuret assay is still frequently used because of its simple analytical procedure and the easy preparation of reagents. Further, compared with other copper-based assays this method is less susceptible to chemical interference (Gupta and Stockham 2014). Moreover, many of the assay kits developed for automated use in wet biochemical analysers, as well as dry chemistry analysers, are based on this principle. The technique is very cheap and this has also contributed to its broad application in veterinary medicine.

The biuret method was modified by using the folin-phenol reagent (Folin-Ciocalteau), which is more sensitive and thus more appropriate to measure low concentrations of proteins (Lowry et al. 1951). In this method, the phenolic groups of tyrosine and tryptophan in proteins react with the Folin-Ciocalteau reagent producing a blue-purple coloured complex (Wilson and Walker 2000). The disadvantages of the Lowry method are the sensitivity to the amino acid composition of the protein and interference by a range of substances, including buffers, drugs, and nucleic acids (Sapan et al. 1999). Another method for the determination of protein concentrations is the Bradford assay, which is based on the binding of the Coomassie brilliant blue dye to the proteins in an acidic solution to form a complex with increased molar absorbance

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(Bradford 1976). This assay is rapid, practical and suitable for simple quantification of proteins in cell lysates, cellular fractions, and recombinant protein samples (Ernst and Zor 2010). It may be performed also in microtitre plates using micro volumes, but its application area is mainly restricted to research laboratories (Da Silva and Zezzi Arruda 2006). Unfortunately, the Bradford assay is only linear over a short range (to 2000 μg/ml) and shows a curvature over this range of protein concentration, which necessitates the dilution of samples before further analysis (Zor and Selinger 1996; Zaia et al. 2005).

Physical methods are also available to measure the protein concentration in serum or plasma. Many veterinary practicers use refractometry for rapid determination of protein concentrations in plasma or other biological fluids. In general, the refractometer measures the degree to which light bends as it passes through the interface between two substances of different densities (George and O'Neill 2001). The angle of refraction changes proportionally to the concentration of solute in solution. Protein is the most important solute dissolved in serum; thus, the refractive index reflects the concentration of proteins in the sample (George 2001). Results obtained from human serum samples showed a good correlation between refractometry and the biuret method (Lien and Wang 1995), but the results for veterinary samples are less consistent. Indeed, whereas some authors have reported a good correlation of results for domestic mammals (biuret methods vs refractometry), others showed either higher or lower values for refractometry compared to the biuret method (Green et al. 1982; Thomas and Brown 1992). The differences between the methods were of magnitudes of 6 g/l and 2 g/l in dogs and cats, respectively (George and O'Neill 2001). However, the most marked differences between the biuret and refractometric methods were observed in avian samples (George 2001). These variations may be caused by differences in the design of various refractometers assigned by the manufacturers, variation in the biuret reagent mixture, as well as assay conditions (Doumas et al. 1981).

2.2. Protein fractionation

For identification and quantification of individual serum proteins or groups of proteins, the proteins in the serum or plasma must either be separated or the individual proteins must be measured independently. Fractionation technologies are among the most important steps in protein analysis, as well as in the discovery of new biomarkers in veterinary medicine. The currently available separation technologies do not allow the analysis of whole protein complexes in a simple step because of the large number of proteins present in serum (Ahmed 2009).

2.2.1. Albumin and globulins

The primary separation of the serum proteins divides complex mixtures into albumin and globulins. Several fractionation techniques have been developed to separate and consequently quantify the proteins in serum (Issaq et al. 2002). Most of them depend on the initial determination of total serum proteins, and then the concentrations of the main fractions are calculated according to the used method. For example, when either the albumin or globulins are determined, the other can be calculated by the subtraction from the total protein concentration (Eckersall 2008). A number of methods have been developed to measure the concentration of globulins. One of these techniques is based on the precipitation of globulins using solutions of metal salts, e.g. sodium sulphite or zinc sulphate (McEwan et al. 1970; Pfeiffer and McGuire 1977). The addition of salts causes turbidity, which may be visually evaluated or measured using a spectrophotometer as units of turbidity. This method may be used as a field test for the evaluation of suckling efficiency or failure of passive transfer of maternal immunity via colostrum in calves and foals (Sedlinska et al. 2005; Hogan et al. 2015). Currently, the bromcresol green (BCG) and bromcresol purple methods are the basis for the determination of serum albumin (Watanabe et al. 2004). Bromcresol green binds quantitatively with albumin forming an intense blue-green complex, and the intensity of the colour produced is directly proportional to the albumin concentration in the sample (Kessler et al. 1997). This method is easy to perform, rapid, and cheap, but less sensitive and selective compared to immunoassays (Doumas and Peters 1997). The reaction between serum and BCG is not specific for albumin; therefore, the BCG method often overestimates the concentrations of serum albumin. However, its specificity can be improved by

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minimising the contact time with the serum sample (Brackeen et al. 1989).

2.2.2. The globulin fractions

The globulins can be subdivided into fractions (α-, β-, and γ-globulins) by electrophoresis, which also allows the quantitative estimation of each fraction from the total protein concentration by proportion. Electrophoresis is the current standard and most widely used fractionation technique for serum proteins in clinical biochemistry and molecular biology (O'Connell et al. 2005). It is based on the movement of charged particles through a support medium soaked in a solution when subjected to an electrical field (Azim et al. 2004). Serum proteins have a negative charge, so in the electrophoretic chamber they migrate toward the positive pole in an electrical field and are separated from each other into different bands according to their sizes (Esmaeilnejad et al. 2014). After separation, the protein fractions are fixed in an acid solution to denature the proteins and immobilise them on the support medium (Tymchak 2010). The proteins are then stained and their quality/quantity is measured by the electrophoretic system which also provides their graphical distribution (Vavricka et al. 2009).

The separation of proteins in an electric field was introduced by Tiselius in the 1930s (Tiselius 1937). The application of serum protein electrophoresis in clinical biochemistry laboratories started in 1950s using paper strips (Turba and Enenkel 1950), which were replaced a few years later by microporous acetate membranes (Kohn 1957). In the 1970s, agarose gel as a support medium was introduced for the electrophoretic separation of proteins (Lehmann et al. 1997), and became the most commonly used supporting substance in veterinary medicine. A major difference between the aforementioned electrophoretic methods is the material of support used for the protein fractionation. According to Luraschi et al. (2003), the protein patterns and the numbers of peaks are dependent on the material used for support, i.e. cellulose acetate vs agarose gel electrophoresis. Standard agarose gel electrophoresis is a labour-intensive method. However, the introduction of pre-packaged gels and the development of new equipment has allowed the automatization of this procedure (Karcher and Nuttall 2001; Ceron et al. 2010). Moreover, it has several advantages compared to cellulose acetate. Indeed, agarose gel as a support medium provides better resolution, higher reproducibility of results and greater clarity of the electrophoretic bands (Riond et al. 2009).

In past few years, capillary zone electrophoresis (CZE) is being used also in veterinary laboratories (Crivellente et al. 2008). In CZE, separation of protein fractions occurs in a free liquid medium created by the low viscosity buffer, in which the high voltage applied generates an electro-osmotic flow that forces the proteins to rapidly move toward the cathode (Giordano and Paltrinieri 2010). This allows better separation of proteins with similar physicochemical characteristics, thus generating multiple sub-peaks or narrower peaks (Petersen et al. 2003). The higher resolution of CZE can often result in abnormal electrophoretic profiles caused by the aforementioned multiple sub-peaks of unknown significance, which is the disadvantage of this method.

Electrophoresis is normally used for serum samples, but plasma or other body fluids (urine, cerebrospinal fluid) may also be processed. Serum is the best material for protein electrophoresis, as it does not contain fibrinogen. Fibrinogen migrates between the β and γ regions on the electrophoretogram, and can influence the correct separation and identification of these fractions (Rossi et al. 2008). Thus, electrophoretic analysis of plasma may provide inaccurate results unless plasma is defibrinated (Errico et al. 2012). On the other hand, in routine practice plasma is often the only sample available, especially in birds and reptiles, in which only small blood specimens can be collected from sick animals (Briscoe et al. 2010). According to Errico et al. (2012), electrophoretic analysis of native plasma provides the same diagnostic information as analysis of serum, except for possible overestimation of the β-globulin fraction. Electrophoretic techniques may also be used for the analysis of urinary proteins, which is a fundamental step in the early diagnosis and subsequent monitoring of renal diseases (Giori et al. 2011). It was found that polyacrylamide gel electrophoresis may localise the origin of urinary proteins based on their molecular weight, providing a diagnostic sensitivity comparable to results obtained by kidney biopsy (Brown et al. 2010). It is considered a very sensitive method to discriminate between glomerular, tubular, or mixed proteinuria (Zini et al. 2004). Agarose gel electrophoresis may be applicable also to separate the main protein fractions in cerebrospinal fluid (CSF) samples. Evaluation of proteins in CSF may provide important information about the

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production of immunoglobulins within the central nervous system, as well as possible disturbances in the blood-brain barrier (Chrisman 1992). However, because of the small amount of proteins in CSF compared with serum, prior concentration of the proteins is necessary to increase the sensitivity of CSF protein detection by electrophoresis (Gama et al. 2007).

2.3. Analysis of specific serum proteins

Recently, high resolution electrophoresis, two-dimensional electrophoresis and proteomic techniques were developed for the separation of proteins. These techniques may be used to simultaneously identify hundreds of proteins and localise specific proteins within several subfractions, whereas agarose gel electrophoresis can separate serum proteins only into five or six fractions (Eckersall 2008). Many specific serum proteins can also be measured by immunoassays, which require a specific antibody against the analysed serum protein. Enzyme immunoassay and enzyme-linked immunosorbent assay (ELISA) are the most common analytical tools in biomedical research for the detection and quantification of specific proteins, antigens or antibodies (Gan and Patel 2013). ELISA is based on the concept of an antigen/protein binding to its specific antibody, which allows detection of very low concentrations of antigen/protein (Mariani et al. 1998). Various types of ELISAs have been developed, all based on the basic principle of the direct or indirect detection of antigen by adhering or immobilising the antigen or antigen-specific capture antibody onto the well surface (Dobrovolskaia et al. 2006). Direct ELISA is the simplest format that uses an antibody directly conjugated to an enzyme to detect plate-immobilised antigen (Lin 2015). The indirect detection method uses a secondary antibody to amplify the signal of the primary antibody bound to the antigen. On the other hand, some serum proteins can be measured on the basis of their biological activities. For example, the concentrations of haptoglobin can be rapidly measured using its high affinity for haemoglobin and subsequent preservation of its peroxidase activity at a low pH (Eckersall et al. 1999). The intensity of the peroxidase activity in the bound haemoglobin is directly proportional to the amount of haptoglobin present in the sample. This colorimetric assay is designed to detect haptoglobin in a range of animal species. The concentrations of ceruloplasmin, another serum protein, can be analysed by measuring its endogenous oxidase activity (Ceron and Martinez-Subiela 2004). However, for the quantitative determination of most serum proteins in animals, species-specific assays still need to be developed.

3. Normal serum protein pattern

Following electrophoresis, serum proteins can be separated into four basic fractions including albumin, α-, β- and γ-globulins (Bossuyt 2006; Figure 1). Each band is made up of a group of individual proteins, each of which is characterised by independent metabolic properties (Table 1). The interpretation of serum protein electrophoretic patterns depends on the variations among different species of animals, as well as among different groups of animals (Figure 2). Indeed, many researchers have noted that serum protein fractions display important differences among all domestic animals (Keay and Doxey 1981; Alberghina et al. 2010). The number, shape, and size of fractions and subfractions vary greatly with the animal species and breed (Fayos et al. 2005): the most important differences are between β-globulins and also γ-globulins.

Protein electrophoresis is also an important laboratory technique for the evaluation of serum protein abnormalities in dogs and cats (McGrotty and Knottenbelt 2002). Using agarose gel electrophoresis Abate et al. (2000) determined six fractions in healthy dogs: albumin, α_1-, α_2-, β_1-, β_2-, and γ-globulins. Similar findings were reported by Harrus et al. (1996), Fayos et al. (2005) and Maciel et al. (2012). In contrast, Akdogan Kaymaz et al. (1999) separated the serum proteins only into five

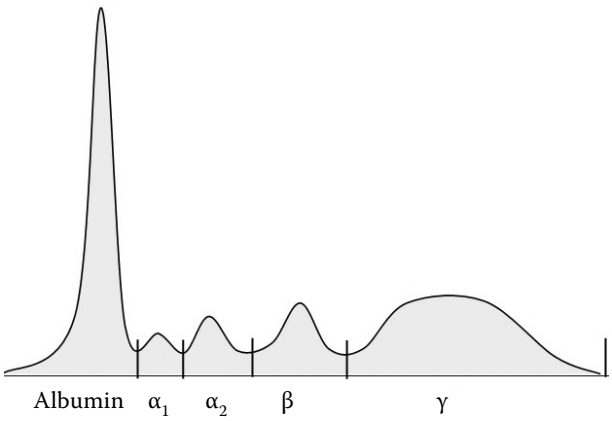

Figure 1. Normal serum protein electrophoretogram

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Table 1. Protein components identified by serum protein electrophoresis (adapted from Tymchak 2010)

Protein fraction	Components
Prealbumin	prealbumin (transthyretin)
Albumin	albumin
α_1-globulins	α_1-antitrypsin
	α_1-fetoprotein
	α_1-acid glycoprotein (orosomucoid)
	α_1-antichymotrypsin
	α_1-lipoprotein
α_2-globulins	haptoglobin
	α_2-microglobulin (protein HC)
	α_2-macroglobulin
	ceruloplasmin
	retinol-binding protein
	α_2-antiplasmin
	α_2-lipoprotein
	Gc-globulin (group specific component, vitamin D-binding protein)
β-globulins	transferrin
	ferritin
	β_1-lipoprotein
	haemopexin
	plasminogen
	angiostatin
	pregnancy-specific β_1-glycoprotein
	β_2-microglobulin
	C3 complement
	C4 complement
	C-reactive protein
	fibrinogen (in plasma protein electrophoresis)
γ-globulins	immunoglobulin G
	immunoglobulin A
	immunoglobulin M
	immunoglobulin D
	immunoglobulin E

fractions, consisting of albumin, α_1-, α_2-, β-, and γ-globulins. The electrophoretic pattern of serum proteins in cats is poorly characterised. In routine electrophoresis, serum cat proteins are separated into albumin, α_1-, α_2-, β_1-, β_2-, and γ-globulin fractions (Baker and Valli 1988). However, according to Gerou-Ferriani et al. (2011), the globulin fractions in normal cats may be further divided into α_{1a}-, α_{1b}-, α_{2a}-, α_{2b}-, β_1- and β_2-subzones.

In healthy horses, some authors (Kohn 1957; Riond et al. 2009; Gundasheva 2015) clearly separated on the serum protein electrophoretogram six different bands, including albumin, α_1- and α_2-globulins, β_1- and β_2-globulins, and γ-globulins. In contrast, Matthews (1982) found two γ-globulin zones. The pig electrophoretic serum protein pattern is not well described. Swindle and Sistino (2015) described five swine protein fractions, comprising albumin, α_1-, α_2-, β-, and γ-globulins.

Differences in the electrophoretic mobility of serum proteins have been observed also between ruminant species. Using agarose gel electrophoresis Nagy et al. (2015) described six fractions in bovine serum comprising albumin, α_1- and α_2-, β_1- and β_2-, and γ-globulins, whereas, Alberghina et al. (2011) and Piccione et al. (2012a) separated the bovine serum proteins into five fractions, comprising albumin, α_1-, α_2-, β-, and γ-globulins. The number of protein fractions in sheep and goat serum varied between various authors. Nagy et al. (2015) and Esmaeilnejad et al. (2014) detected albumin, α_1-, α_2-, β-, γ_1- and γ_2-globulins in sheep serum, while the goat serum proteins showed albumin, α_1-, α_2-, β- and γ-globulin fractions (Perez et al. 2003; Nagy et al. 2015). In contrast, Cyrillo et al. (2006), Fernandez et al. (2006) and Alberghina et al. (2010) determined only one α-globulin and two β-globulin fractions in goat serum.

Serum protein electrophoresis may be a useful diagnostic tool also in avian medicine. It is recommended for many avian species as one of the more reliable techniques to determine the albumin and globulin concentrations, since birds with apparently normal protein values may show abnormalities on the protein electrophoretic pattern (Lumeij et al. 1990). In avian practice, heparinised plasma is the preferred sample for clinical chemistry. Thus, most reports on avian electrophoresis have used plasma instead of serum. There are more variations in protein electrophoretic patterns among avian species than among those of mammals (Werner and Reavill 1999). For example, in avian species β- and γ-globulins migrate in a single fraction with sharp peaks when their concentrations are increased, while in mammals the elevated globulin fractions show broader peaks (Spano et al. 1988). However, many psittacine species have unique protein electrophoretic patterns showing a biphed (twin) β-fraction in healthy, as well as diseased individuals (Cray and Tatum 1998). In most avian species, the

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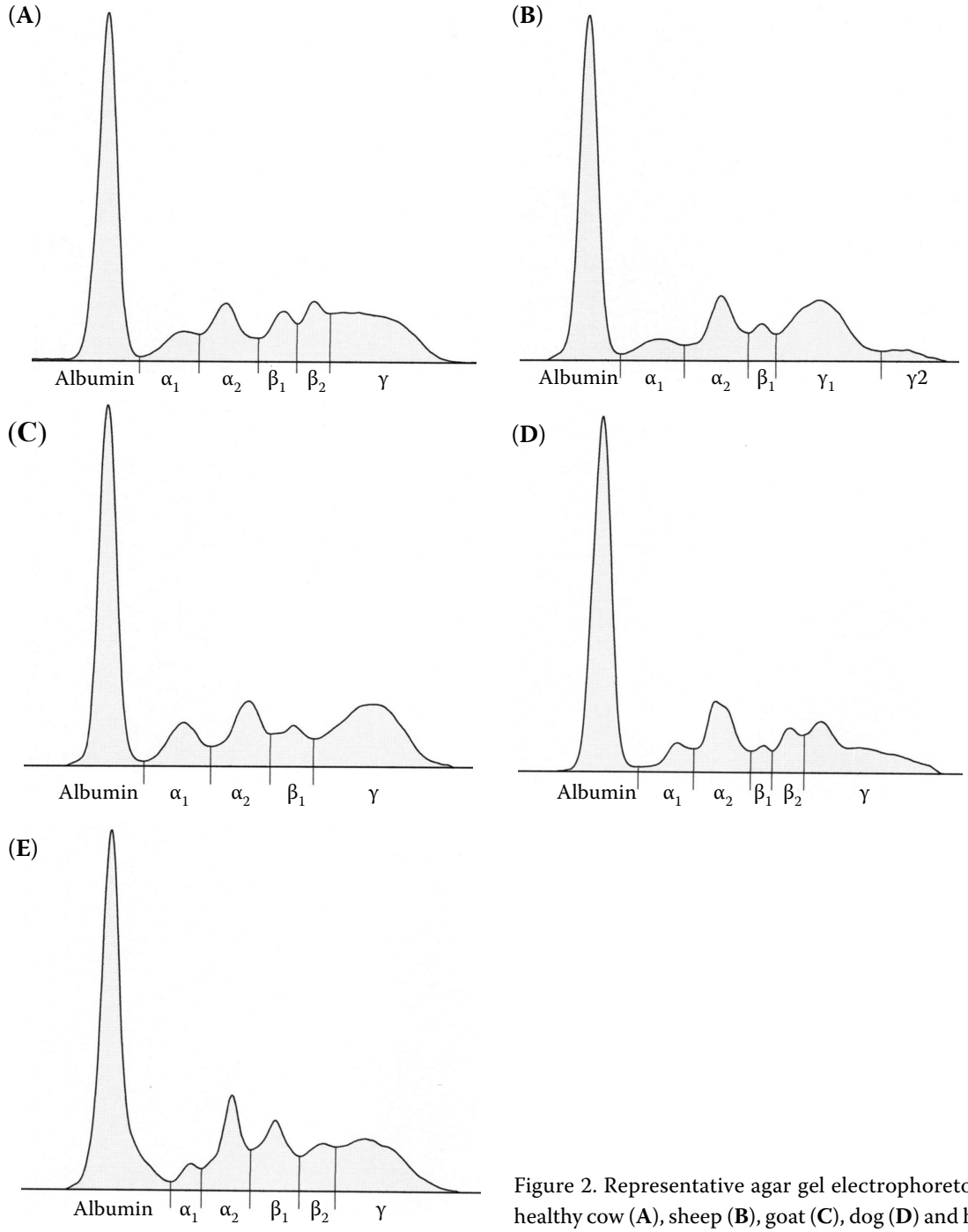

Figure 2. Representative agar gel electrophoretogram in healthy cow (**A**), sheep (**B**), goat (**C**), dog (**D**) and horse (**E**)

α_1-globulin fraction is detectable only in some disease conditions, usually in systemic inflammation. Selective increases in the α_1-globulins have been observed in psittacine birds with parasitic infection, caused by an increase in α_1-antitrypsin (Cray 1997). Wide variations exist also in the prealbumin concentrations, which account for as little as 10% of the total albumin concentration in cockatiels (*Nymphicus hollandicus*) and as much as 75% of the total in budgerigars (*Melopsittacus undulatus*, Cray and Tatum 1998).

3.1. Prealbumin (transthyretin)

Prealbumin is the most rapidly migrating protein fraction in serum and is visible as a band anodic to the main albumin fraction (Harris and Kohn 1974).

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However, it is not always visualized in electrophoretograms and may not exist in all animal species (Kaneko 1997). Prealbumin (transthyretin [TTR]), is a negative acute phase protein, serum concentrations of which fall due to decreased synthesis in response to inflammation and stress, as well as in conditions associated with protein malnutrition (Hamilton and Benson 2001; Beck and Rosenthal 2002). This protein is secreted mainly by the liver and is involved in the transport of both thyroid hormones, and is also indirectly implicated in the carriage of vitamin A through the mediation of the retinol-binding protein (Ingenbleek and Young 1994). Serum TTR concentrations are affected by many factors, including age, gender, and blood-drawing methods, as well as factors influencing distribution or rates of synthesis and catabolism. Assaying serum TTR concentration is recommended by some investigators as a screening marker for inflammation, malnutrition, or both (Johnson et al. 2007). In animals, there are only scant literature data about this protein as a biomarker of health state and its use in laboratory diagnostics (Tothova et al. 2016b).

3.2. Albumin

Albumin is often considered as the only discrete and homogenous protein fraction discernible on the electrophoretogram. In animals, it constitutes between 35 and 50% of total serum protein (Kaneko 1997). The shape and size of the albumin fraction is very similar in all ruminant species reflecting its high serum concentration, homogenous electric charge and high staining affinity, but differences may be found in its relative concentrations between different animal species (Keren 2003). It forms the largest peak and lies closest to the anode on the left side of the panel (Vavricka et al. 2009). Albumin plays an important role in maintaining homeostasis, in transport of substances, and acts as a free-radical scavenger (Hankins 2006). Albumin is responsible for about 75% of the osmotic pressure of plasma and is a major source of amino acids that can be utilised by the animal's body when necessary (Mackiewicz 1997). Serum albumin is the major negative acute phase protein. During the acute phase response the amino acid demand for synthesis of the positive acute phase proteins is markedly increased, which necessitates reprioritisation of the hepatic protein synthesis. Thus, albumin synthesis is down-regulated and amino acids are shunted toward the synthesis of the positive acute phase proteins (Aldred and Schreiber 1993). Catabolism of albumin occurs in various tissues, where it enters cells by pinocytosis and is then degraded by proteases (Evans 2002). Muscle, liver, and kidney are the main sites of albumin catabolism. The turnover of albumin differs with species and is related to the body size. The half-time for clearance of albumin varies from 1.9 days in the mouse to 14–16 days in ruminants (Prinsen 2004).

3.3. Globulins

The globulins are a very heterogenous group of proteins forming peaks on the right side of the electrophoretic panel. Depending on the species, there may normally be one or two α, one or two β, and one or two γ fractions (Kaneko 1997).

3.3.1. The α-globulins

The α fraction is the most rapidly migrating protein of all the globulins, and in most species it migrates as an α_1 (fast) and an α_2 (slow) fraction. Many diagnostically important acute phase proteins migrate in this fraction. α_1-antitrypsin, α_1-acid glycoprotein, α_1-antichymotrypsin, α_1-fetoprotein, and α_1-lipoprotein have been identified in the α_1-globulin fraction, while haptoglobin, α_2-microglobulin, α_2-macroglobulin, ceruloplasmin, α_2-antiplasmin and α_2-lipoprotein have been detected in the α_2-globulin fraction (Bossuyt 2006; Tothova et al. 2014). Acute phase proteins are a large and varied group of plasma proteins with numerous differences in their concentrations between different animal species (Eckersall and Bell 2010). Their concentrations increase in response to any changes in homeostasis or tissue injury. They have specific functions in the regulation of inflammatory processes, predominantly at the site of inflammatory lesions, but they may also act systemically (Gabay and Kushner 1999). In general, the main function of the acute phase proteins is to defend the host against pathological damage, assist in the restoration of homeostasis and in the regulation of different stages of inflammation (Petersen et al. 2004). Moreover, some proteins from these fractions may act as inhibitors

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of enzymes, as digest proteins, as compounds of the blood coagulation system or as carriers of copper (O'Connell et al. 2005).

3.3.2. The β-globulins

The β-globulins are a group of globular proteins that are more mobile than γ-globulins in electrically charged solutions, but less mobile than α-globulins. Transferrin and complement are the main proteins in the β-globulin fraction, which may correspond to the two subfractions (β_1 and β_2) identified in some animal species (Ceron et al. 2010). Other important proteins in this fraction are β_2-microglobulin, C-reactive protein, ferritin, haemopexin, plasminogen, and angiostatin. Moreover, in response to different antigenic stimulations, some IgM immunoglobulins may migrate in the β region, while the IgA and IgE immunoglobulins can be found in the β-γ interzone, which may also correspond to the β_2 subfraction identified in some animal species (Meyer and Harvey 2004).

3.3.3. The γ-globulins

The γ-globulin fraction is predominantly composed of immunoglobulins of various classes (IgG, IgA, IgM, IgD and IgE). While in some animal species (cattle and goats) the γ-globulins migrate as one overall fraction, in sheep they may be separated in two sub-fractions (γ_1 and γ_2; Nagy et al. 2015). According to Kaneko (1997), immunoglobulins from the γ fraction may migrate rapidly or slowly, giving rise to these two sub-fractions. On the other hand, Vavricka et al. (2009) stated that some immunoglobulin classes may migrate into the β-γ zone or β-region. Immunoglobulins (or antibodies) function as a part of the body's immune system which responds to stimulation by molecules recognised by the body as non-self, the so-called antigens. They have the ability to specifically bind to one or a few closely related antigens in order to mediate their neutralisation and elimination, which is their primary function in the protection of the host (Schroeder and Cavacini 2010). Immunoglobulins are produced by cells of the adaptive immune system, activated B cells and plasma cells, in response to the exposure to antigens (Jackson and Elsawa 2015).

4. Serum protein pattern variations related to non-pathological conditions

Many pathological conditions are associated with slight or more marked alterations in serum protein patterns, such as shifts in albumin and globulin concentrations (Keay and Doxey 1982). On the other hand, variations in the serum protein profile and concentrations may occur also under some physiological conditions (Weaver et al. 2000; Janku et al. 2011).

Animal age is one of these important factors that may affect the concentrations of the different serum protein fractions or their electrophoretic pattern (Fayos et al. 2005). That has been shown both in feline species and horses (Baker and Valli 1988; Paltrinieri et al. 2008) and in young and adult cattle (Tothova et al. 2013a), where the most important age-related differences were related to the concentrations of α- and γ-globulins. Indeed, in the latter the concentrations of α_1-globulins were higher in calves, whereas the γ-globulins were higher in adult animals. It has been observed that these important changes happen in the first month of calf life, in association with changes in nutrition and because of adaptation processes during the neonatal period (Tothova et al. 2016a). The concentrations of total proteins and γ-globulins increase rapidly one day after the intake of colostrum, and then decrease gradually until the end of the 1st month of age. Hammon et al. (2002) also stated that at birth, the concentration of total serum proteins of most offspring is very low due to the minimal quantities of immunoglobulins but, that this increases during the first 24 h of life thanks to the intestinal absorption of proteins (particularly immunoglobulins) from colostrum. On the other hand, albumin, the most prominent protein fraction at birth, decreases in relative concentration one day after colostrum intake, with a subsequent gradual increase from Day 2 until the end of the first month of life. At birth, the calf's α_1-globulins comprise almost 30% of total proteins but their concentrations are decreased approximately by 50% at one day after birth, with a further decrease up to Day 30 of life (Tothova et al. 2016a). In the absolute concentrations of α_1-globulins, a temporary slight increase after birth has been observed with a subsequent gradual decrease. Delivery is surely a stressful situation for the offspring, and manifests as higher concentrations of acute phase proteins

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at birth, which migrate into this fraction (Orro et al. 2008). The subsequent increase in the IgG level (due to the colostrum absorption) could substitute for the acute phase response. According to Yuki et al. (2010), most of the acute phase proteins have lower values at birth than in the following days, likely because the liver, which is the main site of production of acute phase proteins, is less mature in new-born than in young or adult animals.

Kaneko (1997) also reported that the large amounts of α-globulins in the blood of new-borns and young animals is due to the higher concentrations of some of the proteins from this fraction (e.g. α_1-fetoprotein), which function to protect young animals from immunological attacks. Similar findings were observed in lambs during the first month of life (Nagy et al. 2014).

Pregnancy and lactation are further factors that may influence the size of albumin and globulin fractions. Variations in the serum protein profile were found in ewes during pregnancy and lactation, as well as in periparturient goats (Janku et al. 2011; Chiaradia et al. 2012; Piccione et al. 2012b). Changes in the concentrations of protein fractions during the last phase of pregnancy and early postpartum in dairy cows are less well documented. Grunberg et al. (2011) observed lower concentrations of total serum proteins in cows around parturition than outside the parturient period and in the following phases of lactation, which may be associated with the transfer of immunoglobulins from the bloodstream to the mammary gland for the synthesis of colostrum (Roubies et al. 2006). Piccione et al. (2011; Piccione et al. 2012b) found increasing values of α-globulins in dairy cows and ewes post-partum, which may be related to the higher concentrations of the acute phase proteins in response to the processes occurring around the time of parturition.

Hormonal changes and stress may also influence the pattern of serum proteins. Piccione et al. (2009) observed changes in the serum protein profile as a response to exercise in athletic horses. Increased concentrations of total proteins were found in horses during exercise, which may be explained by the redistribution of fluid and electrolytes from the vascular compartment to the extracellular fluid space, accompanied by a decrease in blood volume (Fazio 2011). The extent of these changes in the protein profile is related to the degree of training, as well as to the type and intensity of the exercise (Janicki et al. 2013).

5. Pathological serum protein pattern

A wide variety of diseases can cause changes in the serum protein pattern (Table 2).

Higher concentrations of some acute phase proteins were found in cows with subclinical endometritis, as well as in cows with uterine bacterial contamination (Sheldon et al. 2001; Brodzki et al. 2015). Serum protein electrophoresis is a very important technique to evaluate these abnormalities and to characterise the nature of the hyperproteinaemia or hyperglobulinaemia (Keren et al. 1999). Protein electrophoresis may be very useful when routine investigations are not effective for making medical decisions, providing the basis for further specific laboratory analyses (Kaneko 1997; Mallard et al. 1998).

5.1. Albumin

A decrease in the concentration of albumin is one of the most frequently occurring types of dysproteinaemias. Hypoalbuminaemia can be caused by a decreased hepatic production due to liver diseases such as chronic hepatitis, cirrhosis or liver failure (Lee 2012). Hypoalbuminaemia may also be present in renal diseases and nephrotic syndrome, in which there is an increased loss of this protein in urine caused by glomerular damage (Grauer 2005; Kodner 2009). Moreover, low albumin concentrations may indicate chronic malnutrition, inadequate protein intake, or might be associated with gastrointestinal diseases, internal parasitism and protein-losing enteropathy (Don and Kaysen 2004; Diogenes et al. 2010). On the other hand, serum albumin is the major negative acute phase protein and its synthesis may be markedly reduced during the acute phase response (Jain et al. 2011).

Rarely, a serum protein anomaly called bisalbuminaemia may be observed on the electrophoretogram. Bisalbuminaemia is characterised by the occurrence of a bicuspid electrophoretic pattern in the albumin fraction, where albumin produces two heads (bands of equal or unequal intensity; Chhabra et al. 2013). In this abnormality, albumin may either have increased (fast type variants), or decreased electrophoretic mobility (slow type variants; Kobayashi et al. 1995). In humans, bisalbuminaemia has been described in some pathological conditions, including chronic renal diseases, nephrotic syndrome, diabetes mellitus, pancreatic

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Table 2. Abnormalities in serum protein electrophoretic patterns and associated diseases (adapted from Werner and Reavill 1999)

Protein fraction	Alteration	Associated disease condition
Albumin	decrease	liver disease, chronic hepatitis, cirrhosis, liver failure
		renal diseases, nephrotic syndrome
		chronic malnutrition
		gastrointestinal diseases, protein-losing enteropathy, internal parasitism
		acute inflammation (acute phase response)
	increase	dehydration
α_1-globulins	decrease	α_1-antitrypsin deficiency
		malnutrition, hepatic insufficiency
	increase	active inflammation
α_2-globulins	decrease	haemolytic anaemia
	increase	inflammatory diseases, trauma
		nephrotic syndrome
β-globulins	decrease	malnutrition
		hepatic insufficiency
	increase	inflammatory diseases, infections
		active hepatitis, chronic liver diseases, liver cirrhosis
		nephrotic syndrome
		hypercholesterolemia
		iron deficiency anaemia
γ-globulins	decrease	foetal or pre-colostral sera
		recurrent infections, immune deficiency
		hereditary immune deficiency diseases
	increase	monoclonal – multiple myeloma, chronic lymphatic leukaemia, lymphosarcoma, plasma cell myeloma, malignant lymphoma
		polyclonal – reactive inflammatory conditions, chronic inflammatory processes, severe infections, immune-mediated disorders

disease or Alzheimer's disease (Ejaz et al. 2004; Shetty et al. 2007). In animals, bisalbuminaemia was found in female bottlenose dolphin (*Turiops truncatus*), but it was not associated with disease (Medway 1979). According to Vavricka et al. (2009), the presence of bisalbuminaemia may be caused by increased mobility of albumin due to its binding to bilirubin, non-esterified fatty acids, penicillin or acetylsalicylic acid. The horses may exhibit a minor post-albumin fraction, which frequently increases in animals with hypoalbuminaemia or acute inflammatory diseases, and it is consistently present in chronic liver diseases (Johns 2015).

An increased concentration of albumin in the serum is called hyperalbuminemia, and may be observed in cases of severe dehydration. However, hyperalbuminemia was recorded also in dogs with hepatocellular carcinoma (Cooper et al. 2009).

5.2. Globulins

Increases in the globulin fractions belong to the common findings on serum protein electrophoretograms. Since many acute phase proteins migrate in the α region, the concentrations of α_1- and α_2-globulins may be elevated in acute, as well as chronic inflammatory diseases as a result of the activation of the host inflammatory responses (O'Connell et al. 2005). Increased α-globulins (predominantly α_1-globulins) were found in sheep naturally infected with *Babesia ovis*, as well as in calves affected by respiratory diseases (Apaydin and Dede 2010; Tothova et al. 2013b). The α_2-globulin fraction typically increases in patients with nephrotic syndrome as a result of the increased synthesis of α_2-macroglobulin that migrates in this fraction. Because of its size, the α_2-macroglobulin is unable to pass through glo-

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meruli and therefore it remains in the bloodstream (de Sain-van der Velden et al. 1998). Increases in α_2-globulins have been reported in small animals affected by various diseases, including leishmaniasis, ehrlichiosis, babesiosis, feline infectious peritonitis, feline cholangitis, haemoplasmosis, as well as any secondary inflammation, especially if caused by opportunistic bacteria (Camacho et al. 2005; Tappin et al. 2011). Decreases in the α_1-globulin fraction may be detected in α_1-antitrypsin deficiency, a rare genetic disorder in humans and even more rare in animals (Slev et al. 2008). Similarly, the α_2-globulin zone may typically be decreased in haemolytic anaemia, when haptoglobin from this fraction binds with the free haemoglobin released from the destroyed red blood cells, forming haptoglobin-haemoglobin complexes that are rapidly removed by phagocytes (Vavricka et al. 2009; Gupta et al. 2011). On the other hand, the inflammatory conditions that develop in association with haemolytic anaemia lead to increased haptoglobin concentrations that may induce an increase in α_2-globulins (Tecles et al. 2005).

Inflammatory diseases and infections may be accompanied not only by increased concentrations of α-globulins, but also by elevations in the β-fraction as a result of the increased production of some acute phase proteins which migrate into this region. According to Kaneko (1997), increases solely in the β-globulin fraction are not frequent and may be typical for active hepatitis. Chronic persistent liver disease, liver cirrhosis, as well as nephrotic syndrome may cause elevations in the β-region due to the increase of the concentrations of β_2-microglobulin in these conditions (Revillard 1980; Joven et al. 1997). Higher concentrations of β_1-globulins were found also in horses with larval cyathostomiasis (Kent 1987; Mair et al. 1993). High β-globulin concentrations may be associated also with hypercholesterolemia, which is caused by an increased concentration of beta-lipoproteins in this fraction (Pagana and Pagana 2006). Moreover, increased levels of β-globulins are typical for iron deficiency anaemia associated with higher values of transferrin (Firkin and Rush 1997; Killip et al. 2007). The increase of β-globulins in haemolytic anaemia may depend on the presence of free haemoglobin that typically migrates in this region. On the other hand, malnutrition is often accompanied with decreased concentrations of β-globulins.

In some conditions, the increase in the β_2- and γ-globulin fractions may produce a β-γ fusion. This phenomenon is called β-γ bridging and is characterised by the lack of a clear demarcation between these two fractions. It is caused by an increase in the concentrations of IgM or IgA, which may migrate in the region between the β and γ zones (Morris and Johnston 2002). According to some authors, the pattern of β-γ bridging is pathognomonic for chronic liver diseases or hepatic cirrhosis (Evans and Duncan 2003). However, Camus et al. (2010) claimed that β-γ bridging does not have a strong predictive value for hepatic diseases in dogs, cats, or horses, and may be frequently found in association with infectious diseases, including leishmaniasis or ehrlichiosis (Romdane et al. 1992). Another possible source of the β-γ bridge is the use of plasma instead of serum, where it is caused by the migration of fibrinogen between the β and γ regions (Rossi et al. 2008).

Increases in γ-globulin production are called gammopathies. They are very frequent and may be found in many pathological conditions. There are two different types of gammopathies: monoclonal and polyclonal gammopathy. Monoclonal gammopathy is characterised by a sharp, homogenous, spike-like peak in the focal region of the γ-globulin zone (Figure 3). This reflects the presence of excessive amounts of one type of immunoglobulin secreted by a single clone of B lymphocytes, or an immunoglobulin fragment referred to as paraprotein or M protein (Edwards et al. 1993). Multiple myeloma is the most common malignant disorder of plasma cells, in which usually IgA and IgG paraproteins can be found (Weiss et al. 2009). However, it is important to differentiate multiple myeloma from monoclonal gammopathy of undetermined significance, which is a premalignant plasma cell dis-

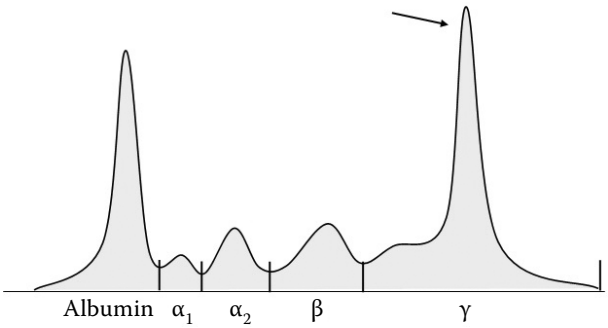

Figure 3. Electrophoretogram of individual with a monoclonal gammopathy. A narrow peak (arrow) is present in the γ-region

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order present in more than 3% of the general human population at the age of 50 years and older (Kyle et al. 2006). Other causes of monoclonal gammopathy are chronic lymphatic leukaemia, lymphosarcoma, or Waldenstrom's macroglobulinaemia, where IgM paraproteins can be found (Kyle 1994). Monoclonal gammopathies in farm animals are not frequent. Some cases were recorded in horses, which has been associated with plasma cell myeloma, malignant lymphoma and idiopathic causes (Kent and Roberts 1990; Edwards et al. 1993). Reports on mono- or oligoclonal gammopathy in small animals are more numerous, and were found in cases with erythrophagocytic multiple myeloma, Waldenstrom's macroglobulinaemia, as well as chronic canine ehrlichiosis with persistently increased antibody titres (Perille and Matus 1991; Webb et al. 2008; Jaillardon and Fournel-Fleury 2011). Biclonal gammopathy has also been described in a cat and in a dog with plasma cell neoplasia and light-chain multiple myeloma (Yamada et al. 2007; Facchini et al. 2010).

Polyclonal gammopathy reflects the presence of a diffuse hypergammaglobulinaemia, in which all immunoglobulin classes may be increased. It is characterised by a diffuse, broad increase in the γ-globulin zone on the electrophoretogram (Figure 4). This elevation of γ-globulins usually indicates a non-malignant condition, and is mostly caused by reactive and inflammatory processes (O'Connell et al. 2005). The most common causes of polyclonal gammopathies are chronic inflammatory processes (gastrointestinal, respiratory, endocrine, cardiac), severe infections, as well as immune-mediated or rheumatological disorders (Dispenzieri et al. 2001; Crisman et al. 2008; Vavricka et al. 2009). Polyclonal gammopathy may be typically described in animals with leishmaniasis, feline infectious peritonitis, feline leukaemia virus, as well as *Ehrlichia canis* infection (Tappin et al. 2011; Baba et al. 2012). A decrease in the concentrations of γ-globulins in the serum is called hypogammaglobulinaemia. This pattern is typical for foetal or pre-colostral sera in some animal species. In calves and foals, pre-colostral serum normally contains no or only very small quantities of γ-globulins, but within a few hours after the intake of colostrum, γ-globulins begin to rise in serum and the absorption continues for up to 36 h after birth, after which gut permeability ceases (Weaver et al. 2000; Blum 2006). Hypogammaglobulinaemia may be commonly seen also in patients with recurrent infections or in cases of immune deficiency (Cunningham-Rundles and Knight 2007). The absence of γ-fraction or very low concentrations of γ-globulins are called agammaglobulinaemia. The condition may be found in people with hereditary immune deficiency diseases, in which the precursor cells that produce gammaglobulins are not sufficiently developed, resulting in low numbers of mature B lymphocytes (B cells; Ochs and Smith 1996). Primary immunodeficiency disorders may be found also in animals, especially in horses (Arabian, Appaloosa) and in some canine breeds, including Basset hounds, Jack Russell terriers, Beagles, German shepherds, Chinese shar-pei, Cardigan Welsh corgi, Doberman pinschers, and Irish setters (Stockham and Scott 2002). Agammaglobulinaemia in adult animals is a rare condition. Primary X-linked agammaglobulinaemia caused by impaired B cells was described in young male horses, and resulted in low immunoglobulin production, marked depletion of the γ-globulin fraction on the electrophoretogram and consequently in clinical manifestation of the disease at the age of 4–5 months (Perryman et al. 1983).

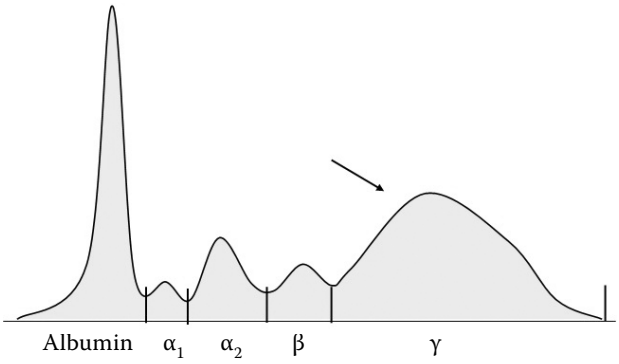

Figure 4. Electrophoretogram of an individual with a polyclonal gammopathy. A broad-based peak (arrow) is present in the γ-region

The aforementioned shifts in the concentrations of albumin and globulins lead also to changes in the albumin: globulin ratio (A/G). The normal A/G ratio ranges from values lower than 1.0 to values just above 1.0 (e.g. 0.6–1.1 in dogs and cats, 0.6–1.4 in horses, 0.6–0.9 in cows; Krimer 2011), but many disease conditions may alter the relative concentrations of albumin and globulins, changing their proportion (Kaneko 1997). A decreased A/G ratio may be caused by the overproduction of globulins, decreased synthesis of albumin, or due to the loss of albumin from the circulation. On the other hand, a higher A/G ratio reflects the underproduction of globulins. Thus, the A/G ratio provides very important information about the changes in the electrophoretic pattern, and

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could help in the classification and identification of dysproteinaemias (Alberghina et al. 2011).

6. The usefulness of serum protein electrophoresis in clinical practice

The analysis of serum proteins and their electrophoretic separation has been extensively used in human medicine for many years. In clinical practice, the main area of the application of serum protein electrophoresis is in the identification of patients with multiple myeloma and other disorders of serum proteins (Vavricka et al. 2009). Moreover, the electrophoretic separation of serum proteins is very important in the differentiation of monoclonal gammopathies from polyclonal gammopathies. For gastroenterologists, the method may be of special interest in the diagnosis of chronic liver diseases, hepatic cirrhosis, hepatocellular carcinoma, α_1-antitrypsin deficiency, inflammatory bowel disease, or amyloidosis, in which clinical signs and symptoms may be seen in the whole gastrointestinal tract (Melmed 2009). In small animals, protein electrophoresis is commonly used to support a clinical diagnosis of diseases characterised by dysproteinaemia (leishmaniasis, ehrlichiosis, feline infectious peritonitis), or to identify the presence of inflammation with increased α-globulins.

Serum protein electrophoresis has been studied intensively also in small animal and equine medicine. Fayos et al. (2005) evaluated the electrophoretic pattern of serum proteins in healthy retired racing Greyhounds in comparison to non-Greyhound dogs of the same age and gender, and they suggested that specific reference intervals should be determined for the parameters related to protein profiles in different breeds of dogs. The serum protein responses, including the concentrations of separated protein fractions were determined in dogs with naturally occurring mild, severe or complicated *Babesia canis* infection (Lobetti et al. 2000). These authors found lower concentrations of total proteins, albumin and α-globulins in dogs with mild and severe babesiosis, while the dogs with complicated babesiosis showed no typical serum protein pattern. Akdogan Kaymaz et al. (1999) evaluated the usefulness of serum protein electrophoresis in the differentiation of gastrointestinal parasitic infections caused by taeniosis, coccidiosis, ancylostomosis, trichuriosis and ascaridiosis in dogs. In animals with coccidiosis significantly lower concentrations of α_1-globulins were found, while the dogs with ancylostomosis had higher values of α_2-globulins. On the other hand, the α_2-globulin fraction decreased significantly in the dogs with ascaridiosis. Possible alterations in the serum protein electrophoretic profile after the inoculation of live attenuated vaccine against canine distemper virus and canine parvovirus were studied by Maciel et al. (2012) in puppies. At Day 7 post-vaccination, an increase of the concentrations of α_1- and α_2-globulins was observed. An understanding of the pathophysiological mechanisms initiated by the first two vaccinations is very important, because they can be easy confused with diseases in puppies.

Although serum protein electrophoresis provides useful information on the changes in the concentrations of albumin and globulin fractions associated with pathological conditions, in ruminants it is a rarely used diagnostic tool. The usefulness of the electrophoretic separation of serum proteins was studied by Woolf et al. (1973) in bighorn sheep with chronic pneumonia attributed to *Mycoplasma*. In this study, diseased sheep had significantly lower albumin, and higher α_1- and γ-globulin concentrations. Recently, Tothova et al. (2013b) evaluated the effect of chronic bronchopneumonia on the serum protein pattern in calves. These authors found significantly higher concentrations of α_1-, β_2- and γ-globulins in the affected animals compared with healthy ones. Yoshida (1991) investigated the diagnostic significance of protein electrophoresis in cows with traumatic pericarditis. In the affected cows slight hypoproteinaemia, moderate hypoalbuminemia, and a slight increase in the concentration of the α- and β-globulin fractions was observed. A tendency toward hypergammaglobulinaemia was found in cows with purulent pericarditis, while a large indentation between the β- and γ-fractions was typical for fibrinous or sero-fibrinous pericarditis. Changes in the serum electrophoretic pattern and immunoglobulin concentrations were examined also in cows with lymphoma (Jacobs et al. 1980). Moderately increased concentrations of α_2-globulins were found in these cows, while the β_2-globulin fraction was significantly decreased due to the lower concentration of immunoglobulins. Alterations of the serum protein electrophoretic profile were investigated also in sheep naturally infected with *Babesia ovis* (Apaydin and Dede 2010). The results of this study suggested

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a marked decrease of both total serum proteins and all protein fractions in diseased sheep before treatment compared to healthy animals. The concentrations of total protein and globulins (except for the α-globulin fraction) increased significantly five days after treatment, but the values were still lower than those measured in healthy sheep. The aforementioned authors stated that babesiosis may induce intense proteolysis of the circulating proteins probably due to the altered protein synthesis by the liver, which was improved by the eradication of parasites. Similarly, Diogenes et al. (2010) evaluated whether the inflammatory reactions caused by infection with internal parasites like *Haemonchus contortus* may induce alterations in the serum protein profile in goats. The results of this study showed a severe hypoproteinaemia and hypoalbuminemia in infected goats, while the concentrations of α- and γ_2-globulins were markedly increased.

7. Conclusions

The aforementioned data suggest that serum protein electrophoresis may be a useful diagnostic tool also in veterinary medicine. The electrophoretic technique may provide clinicians with important information to facilitate the differentiation of dysproteinaemias and paraproteinaemias.

Changes in serum proteins can be indicative of non-specific pathological processes or may represent potential diagnostic markers of some pathological conditions. The determination of the physiological electrophoretic patterns in all animal species could be very useful for clinicians in differentiating healthy from sick animals, and may provide a basis for further specific laboratory investigations. Therefore, a main challenge in dealing with serum protein electrophoresis of domestic animals is gaining a clear picture of the differences in the normal physiological pattern between species. Only subsequently can an assessment of the diagnostic significance of the changes in protein fractions be made.

8. REFERENCES

Abate O, Zanatta R, Malisano T, Dotta U (2000): Canine serum protein patterns using high-resolution electrophoresis (HRE). The Veterinary Journal 159, 150–154.

Ahmed FE (2009): Sample preparation and fractionation for proteome analysis and cancer biomarker discovery by mass spectrometry. Journal of Separation Science 32, 771–798.

Akdogan Kaymaz A, Bakirel U, Gonul R, Tan H, Vurusaner C (1999): Serum protein electrophoresis in dogs with intestinal parasites. Turkish Journal of Veterinary and Animal Sciences 23, 457–459.

Alberghina D, Casella S, Vazzana I, Ferrantelli V, Giannetto C, Piccione G (2010): Analysis of serum proteins in clinically healthy goats (Capra hircus) using agarose gel electrophoresis. Veterinary Clinical Pathology 39, 317–321.

Alberghina D, Giannetto C, Vazzana I, Ferrantelli V, Piccione G (2011): Reference intervals for total protein concentration, serum protein fractions, and albumin/globulin ratios in clinically healthy dairy cows. Journal of Veterinary Diagnostic Investigation 23, 111–114.

Aldred AR, Schreiber G (1993): The negative acute phase protein. In: Mackiewicz I, Kushner I, Baumann H (eds): Acute Phase Proteins. Molecular Biology, Biochemistry, and Clinical Applications. CRC Press, Boca Raton, Florida. 21–37.

Anderson NL, Anderson NG (2002): The human plasma proteome. Molecular and Cellular Proteomics 1, 845–867.

Apaydin B, Dede S (2010): Electrophoretic profile of serum protein fractions from sheep naturally infected with Babesia ovis. Revue de Medecine Veterinaire 161, 57–60.

Azim W, Azim S, Ahmed K, Shafi H, Rafi T, Luqman M (2004): Diagnostic significance of serum protein electrophoresis. Biomedica 20, 40–44.

Baba K, Itamoto K, Amimoto A, Kitagawa K, Hiraoka H, Mizuno T, Sato H, Okuda M (2012): Ehrlichia canis infection in two dogs that emigrated from endemic areas. Journal of Veterinary Medical Science 74, 775–778.

Baker RJ, Valli VEO (1988): Electrophoretic and immunoelectrophoretic analysis of feline serum proteins. Canadian Journal of Veterinary Research 52, 308–314.

Beck FK, Rosenthal TC (2002): Prealbumin: a marker for nutritional evaluation. American Family Physician 65, 1575–1578.

Blum JW (2006): Nutritional physiology of neonatal calves. Journal of Animal Physiology and Animal Nutrition 90, 1–11.

Bossuyt X (2006): Advances in serum protein electrophoresis. Advances in Clinical Chemistry 42, 43–80.

Boyer RF (ed.) (2000): Modern Experimental Biochemistry. 3rd edn. Addison Wesley Longman Inc., California. 480 pp.

Brackeen GL, Dover JS, Long CL (1989): Serum albumin. Differences in assay specificity. Nutrition in Clinical Practice 4, 203–205.

Bradford MM (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing

doi: 10.17221/19/2016-VETMED

the principle of protein-dye-binding. Analytical Biochemistry 72, 248–254.

Briscoe JA, Rosenthal KL, Shofer FS (2010): Selected complete blood cell count and plasma protein electrophoresis parameters in pet psittacine birds evaluated for illness. Journal of Avian Medicine and Surgery 24, 131–137.

Brodzki P, Kostro K, Krakowski L, Marczuk J (2015): Inflammatory cytokine and acute phase protein concentrations in the peripheral blood and uterine washings of cows with subclinical endometritis in the late postpartum period. Veterinary Research Communications 39, 143–149.

Brown JS, Nabity MB, Brock R (2010): Comparison of urine sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) with renal histological findings and clinicopathologic data in dogs with renal diseases. Veterinary Clinical Pathology 3, 556.

Burtis CA, Ashwood ER (eds) (2001): Tietz Fundamentals of Clinical Chemistry. 5th edn. WB Saunders Company, Philadelphia. 1091 pp.

Camacho AT, Guitian FJ, Pallas E, Gestal JJ, Olmeda S, Goethert H, Telford S, Spielman A (2005): Serum protein response and renal failure in canine Babesia annae infection. Veterinary Research 36, 713–722.

Camus MS, Krimer PM, LeRoy BE, Almy FS (2010): Evaluation of the positive predictive value of serum protein electrophoresis beta-gamma bridging for hepatic disease in three domestic animal species. Veterinary Pathology 47, 1064–1070.

Ceron JJ, Martinez-Subiela S (2004): An automated spectrophotometric method for measuring canine ceruloplasmin in serum. Veterinary Research 35, 671–679.

Ceron JJ, Caldin M, Martinez-Subiela S (2010): Electrophoresis and acute phase protein measurement. In: Douglas J, Weiss K, Wardrop J (eds): Schalm's Veterinary Hematology. 6th edn. Blackwell Publishing Ltd., Ames. 1157–1161.

Chhabra S, Bansal F, Saikia B, Minz RW (2013): Bisalbuminemia: A rarely encountered protein anomaly. Journal of Laboratory Physicians 5, 145–146.

Chiaradia E, Avellini L, Tartaglia M, Gaiti A, Just I, Scoppetta F, Czentnar Z, Pich A (2012): Proteomic evaluation of sheep serum proteins. BMC Veterinary Research 8, 66.

Chrisman CL (1992): Cerebrospinal fluid analysis. Veterinary Clinics of North America: Small Animal Practice 22, 781–810.

Cooper ES, Wellman ML, Carsillo ME (2009): Hyperalbuminemia associated with hepatocellular carcinoma in a dog. Veterinary Clinical Pathology 38, 516–520.

Cray C (1997): Plasma protein electrophoresis: An update. Proceedings of the Annual Conference of the Association of Avian Veterinarians. 209–212.

Cray C, Tatum LM (1998): Applications of protein electrophoresis in avian diagnostics. Journal of Avian Medicine and Surgery 12, 4–10.

Crisman MV, Scarratt WK, Zimmerman KL (2008): Blood proteins and inflammation in the horse. Veterinary Clinics: Equine Practice 24, 285–297.

Crivellente F, Bonato M, Cristofori P (2008): Analysis of mouse, rat, dog, marmoset, and human serum proteins by capillary electrophoresis: comparison with agarose gel electrophoresis. Veterinary Clinical Pathology 37, 73–78.

Cunningham-Rundles C, Knight AK (2007): Common variable immune deficiency: reviews, continued puzzles, and a new registry. Immunology Research 38, 78–86.

Cyrillo FC, Leal ML do R, Benesi FJ, Della Libera AMM de P (2006): Dynamics of serum proteins during the estrous cycle of goats bred in Brazil and naturally infected by caprine arthritis encephalitis virus. Revista Arquivos do Instituto Biologico 73, 41–44.

Da Silva MAO, Zezzi Arruda MA (2006): Mechanization of the Bradford reaction for the spectrophotometric determination of total proteins. Analytical Biochemistry 351, 155–157.

De Sain-van der Velden MGM, Rabelink TJ, Reijngoud DJ, Gadellaa MM, Voorbij HAM, Stellaard F, Kaysen GA (1998): Plasma α_2 macroglobulin is increased in nephrotic patients as a result of increased synthesis alone. Kidney International 54, 530–535.

Diogenes PVA, Suassuna ACD, Ahid SMM, Soto-Blanco B (2010): Serum protein electrophoretic profile of goats infected with Haemonchus contortus. Journal of Animal and Veterinary Advances 9, 1603–1606.

Dispenzieri A, Gertz MA, Therneau TM, Kyle RA (2001): Retrospective cohort study of 148 patients with polyclonal gammopathy. Mayo Clinic Proceedings 76, 476–487.

Dobrovolskaia E, Gam A, Slater JE (2006): Competition enzyme-linked immunosorbent assay (ELISA) can be a sensitive method for the specific detection of small quantities of allergen in a complex mixture. Clinical and Experimental Allergy 36, 525–530.

Don BR, Kaysen G (2004): Serum albumin: relationship to inflammation and nutrition. Seminars in Dialysis 17, 432–437.

Doumas BT, Peters Jr T (1997): Serum and urine albumin: a progress report on their measurement and clinical significance. Clinica Chimica Acta 258, 3–20.

Doumas BT, Bayse DD, Carter RJ, Peters Jr T, Schaffer R (1981): A candidate reference method for determination of total protein in serum. I. Development and validation. Clinical Chemistry 27, 1642–1650.

Eckersall PD (2008): Proteins, proteomics, and the dysproteinemias. In: Kaneko JJ, Harvey JW, Bruss ML (eds): Clinical Biochemistry of Domestic Animals. 6th edn. Elsevier Academic Press, California. 117–155.

Eckersall PD, Bell R (2010): Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. The Veterinary Journal 185, 23–27.

doi: 10.17221/19/2016-VETMED

Eckersall PD, Duthie S, Safi S, Moffatt D, Horadagoda NU, Doyle S, Parton R, Bennett D, Fitzpatrick JL (1999): An automated biochemical assay for haptoglobin: prevention of interference from albumin. Comparative Haematology International 9, 117–124.

Edwards DF, Parker JW, Wilkinson JE, Helman RG (1993): Plasma cell myeloma in the horse. A case report and literature review. Journal of Veterinary Internal Medicine 7, 169–176.

Ejaz AA, Krishna M, Wasiluk A, Knight JD (2004): Bisalbuminemia in chronic kidney disease. Clinical and Experimental Nephrology 8, 270–273.

Ernst O, Zor T (2010): Linearization of the Bradford protein assay. Journal of Visualized Experiments 38, 1918.

Errico G, Giordano A, Paltrinieri S (2012): Diagnostic accuracy of electrophoretic analysis of native or defibrinated plasma using serum as a reference sample. Veterinary Clinical Pathology 41, 529–540.

Esmaeilnejad B, Tavassoli M, Asri-Rezaei S, Dalir-Naghadeh B, Pourseyed SH (2014): Evaluation of serum total protein concentration and protein fractions in sheep naturally infected with Babesia ovis. Comparative Clinical Pathology 23, 151–155.

Evans EW, Duncan JR (2003): Proteins, lipids, and carbohydrates. In: Latimer KW, Mahaffey EA, Prasse KW (eds): Duncan and Prasse's Veterinary Laboratory Medicine Clinical Pathology. 4th edn. Blackwell Publishing, Ames. 162–192.

Evans TW (2002): Review article: albumin as a drug: biological effects of albumin unrelated to oncotic pressure. Alimentary Pharmacology and Therapeutics 16, 6–11.

Facchini RV, Bertazzolo W, Zuliani D, Bonfanti U, Caldin M, Avallone G, Roccabianca P (2010): Detection of biclonal gammopathy by capillary zone electrophoresis in a cat and a dog with plasma cell neoplasia. Veterinary Clinical Pathology 39, 440–446.

Fayos M, Couto CG, Iaybik MC, Wellman ML (2005): Serum protein electrophoresis in retired racing Greyhounds. Veterinary Clinical Pathology 34, 397–400.

Fazio F, Assenza A, Tosto F, Casella S, Piccione G, Caola G (2011): Training and haematochemical profile in thoroughbreds and standardbreds, a longitudinal study. Livestock Science 141, 221–226.

Fernandez SY, Jesus EEV, Paule BJA, Uzeda RS, Almeida MAO, Guimaraes JE (2006): Proteinogram of alpine goat naturally infected by gastrointestinal parasites. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia 58, 279–282.

Firkin R, Rush B (1997): Interpretation of biochemical tests for iron deficiency: diagnostic difficulties related to limitations of individual tests. Australian Prescriber 20, 74–76.

Friedrichs WE, Navarijoashbaugh AL, Bowman BH, Yang F (1995): Expression and inflammatory regulation of haptoglobin gene in adipocytes. Biochemical and Biophysical Research Communications 209, 250–256.

Gabay C, Kushner I (1999): Acute-phase proteins and other systemic responses to inflammation. New England Journal of Medicine 340, 448–454.

Gama FGV, Santana AE, de Campos Filho E, da Silva Nogueira CA (2007): Agarose gel electrophoresis of cerebrospinal fluid proteins of dogs after sample concentration using a membrane microconcentrator technique. Veterinary Clinical Pathology 36, 85–88.

Gan SD, Patel KR (2013): Enzyme immunoassay and enzyme-linked immunosorbent assay. Journal of Investigative Dermatology 133, e12.

George JW (2001): The usefulness and limitations of hand-held refractometers in veterinary laboratory medicine: An historical and technical review. Veterinary Clinical Pathology 30, 201–210.

George JW, O'Neill SL (2001): Comparison of refractometer and biuret methods for total protein measurement in body cavity fluids. Veterinary Clinical Pathology 30, 16–18.

Gerou-Ferriani M, McBrearty AR, Burchmore RJ, Jayawardena KGI, Eckersall PD, Morris JS (2011): Agarose gel serum protein electrophoresis in cats with and without lymphoma and preliminary results of tandem mass fingerprinting analysis. Veterinary Clinical Pathology 40, 159–173.

Giordano A, Paltrinieri S (2010): Interpretation of capillary zone electrophoresis compared with cellulose acetate and agarose gel electrophoresis: reference intervals and diagnostic efficiency in dogs and cats. Veterinary Clinical Pathology 39, 464–473.

Giori L, Tricomi FM, Zatelli A, Roura X, Paltrinieri S (2011): High-resolution gel electrophoresis and sodium dodecyl sulphate-agarose gel electrophoresis on urine samples for qualitative analysis of proteinuria in dogs. Journal of Veterinary Diagnostic Investigation 23, 682–690.

Gornall AG, Bardawill CJ, David MN (1949): Determination of serum proteins by means of the Biuret reaction. The Journal of Biological Chemistry 177, 751–766.

Grauer GF (2005): Canine glomerulonephritis: new thoughts on proteinuria and treatment. Journal of Small Animal Practice 46, 469–478.

Green SA, Jenkins SJ, Clark PA (1982): A comparison of chemical and electrophoretic methods of serum protein determinations in clinically normal domestic animals of various ages. The Cornell Veterinarian 72, 416–426.

Grunberg W, Donkin SS, Constable PD (2011): Periparturient effects of feeding a low dietary cation-anion difference diet on acid-base, calcium, and phosphorus homeostasis and on intravenous glucose tolerance test in high producing dairy cows. Journal of Dairy Science 94, 727–745.

doi: 10.17221/19/2016-VETMED

Gundasheva D (2015): Electrophoretic analysis of serum proteins in strenuously trained horses revaccinated against equine herpes virus 4/1 and equine influenza virus. Veterinarija i Zootechnika 69, 18–25.

Gupta A, Stockham SL (2014): Negative interference of icteric serum on a bichromatic biuret total protein assay. Veterinary Clinical Pathology 43, 422–427.

Gupta S, Ahern K, Nakhl F, Forte F (2011): Clinical usefulness of haptoglobin levels to evaluate hemolysis in recently transfused patients. Advances in Hematology, DOI: 10.1155/2011/389854.

Hamilton JA, Benson MD (2001): Transthyretin: a review from a structural perspective. Cellular and Molecular Life Sciences 58, 1491–1521.

Hammon HM, Schiessler G, Nussbaum A, Blum JW (2002): Feed intake patterns, growth performance and metabolic and endocrine traits in calves fed unlimited amounts of colostrums and milk by automate strating in the neonatal period. Journal of Dairy Science 85, 3352–3362.

Hankins J (2006): The role of albumin in fluid and electrolyte balance. Journal of Infusion Nursing 29, 260–265.

Harris RI, Kohn J (1974): The pre-albumin fraction: A useful parameter in the interpretation of routine protein electrophoresis. Journal of Clinical Pathology 27, 986–989.

Harrus S, Waner T, Avidar Y, Bogin E, Peh HC, Bark H (1996): Serum protein alterations in canine ehrlichiosis. Veterinary Parasitology 66, 241–249.

Hogan I, Doherty M, Fagan J, Kennedy E, Conneely M, Brady P, Ryan C, Lorenz I (2015): Comparison of rapid laboratory tests for failure of passive transfer in the bovine. Irish Veterinary Journal 68, 18–27.

Ingenbleek Y, Young V (1994): Transthyretin (prealbumin) in health and disease: Nutritional implications. Annual Review of Nutrition 14, 495–533.

Issaq HJ, Conrads TP, Janini GM, Veenstra TD (2002): Methods for fractionation, separation and profiling of proteins and peptides. Electrophoresis 23, 3048–3061.

Jackson DA, Elsawa SF (2015): Factors regulating immunoglobulin production by normal and disease-associated plasma cells. Biomolecules 5, 20–40.

Jacobs RM, Valli VE, Wilkie BN (1980): Serum electrophoresis and immunoglobulin concentrations in cows with lymphoma. American Journal of Veterinary Research 41, 1942–1946.

Jaillardon L, Fournel-Fleury C (2011): Waldenstrom's macroglobulinemia in a dog with a bleeding diathesis. Veterinary Clinical Pathology 40, 351–355.

Jain S, Gautam V, Naseem S (2011): Acute phase proteins: As diagnostic tool. Journal of Pharmacy and Bioallied Sciences 3, 118–127.

Janicki B, Kochowicz A, Buzala M, Krumrych W (2013): Variability of selected biochemical parameters in young stallions during the 100-day performance test. Journal of Equine Veterinary Science 33, 1136–1141.

Janku L, Pavlata L, Misurova L, Filipek J, Pechova A, Dvorak R (2011): Levels of protein fractions in blood serum of periparturient goats. Acta Veterinaria Brno 80, 185–190.

Johns JL (2015): Alterations in blood proteins. In: Smith BP (ed.): Large Animal Internal Medicine. 5th edn. Elsevier, St Louis. 386–392.

Johnson AM, Merlini G, Sheldon J, Ichibara K (2007): Clinical indications for plasma protein assay: transthyretin (prealbumin) in inflammation and malnutrition. Clinical Chemistry and Laboratory Medicine 45, 419–426.

Joven J, Cliville X, Camps J, Espinel E, Simo J, Vilella E, Oliver A (1997): Plasma protein abnormalities in nephritic syndrome: effect on plasma colloid osmotic pressure and viscosity. Clinical Chemistry 43, 1223–1231.

Kaneko JJ (1997): Serum proteins and the dysproteinemias. In: Kaneko JJ (ed.): Clinical Biochemistry of Domestic Animals. 5th edn. Academic Presss, London. 117–138.

Karcher RA, Nuttall KL (2001): Electrophoresis. In: Burtis CA, Ashwood ER (eds): Tietz Fundamentals in Clinical Chemistry. 5th edn. WB Saunders, Philadelphia. 121–132.

Keay G, Doxey DL (1981): Species characteristics of serum proteins demonstrated after agarose gel electrophoresis. Veterinary Research Communications 5, 263–270.

Keay G, Doxey DL (1982): A comparison of the serum protein electrophoretic patterns of young and adult animals. Veterinary Research Communications 5, 271–276.

Kennedy S (2001): Proteomic profiling from human samples: the body fluid alternative. Toxicology Letters 120, 379–384.

Kent JE (1987): Specific serum protein changes associated with primary and secondary Strongylus vulgaris infections in pony yearlings. Equine Veterinary Journal 19, 133–137.

Kent JE, Roberts CA (1990): Serum protein changes in four horses with monoclonal gammopathy. Equine Veterinary Journal 22, 373–376.

Keren DF (ed.) (2003): Protein Electrophoresis in Clinical Diagnosis. Edward Arnold Ltd., London. 63–108.

Keren DF, Alexanian R, Goeken JA, Gorevic PD, Kyle RA, Tomar RH (1999): Guidelines for clinical and laboratory evaluation of patients with monoclonal gammopathies. Archives of Pathology and Laboratory Medicine 123, 106–107.

Kessler MA, Meinitzer A, Wolfbeis OS (1997): Albumin blue 580 fluorescence assay for albumin. Analytical Biochemistry 248, 180–182.

Killip S, Bennett JM, Chambers MD (2007): Iron deficiency anemia. American Family Physician 75, 671–678.

doi: 10.17221/19/2016-VETMED

Kobayashi S, Okamura N, Kamoi K, Sugita O (1995): Bisalbumin (fast and slow type) induced by human pancreatic juice. Annals of Clinical Biochemistry 32, 63–67.

Kodner C (2009): Nephrotic syndrome in adults: Diagnosis and management. American Family Physician 80, 1129–1134.

Kohn J (1957): A cellulose acetate supporting medium for zone elctrophoresis. Clinica Chimica Acta 2, 297.

Krimer PM (2011): Generating and interpreting test results: test validity, quality control, reference values, and basic epidemiology. In: Latimer KS (ed.): Duncan and Prasse's Vaterinary Laboratory Medicine: Clinical Pathology. 5th edn. Wiley-Blackwell, Chichester. 365–382.

Kyle RA (1994): The monoclonal gammopathies. Clinical Chemistry 40, 2154–2161.

Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Offord JR, Dispenzieri A, Katzmann JA, Melton LJ (2006): Prevalence of monoclonal gammopathy of undetermined significance. New England Journal of Medicine 354, 1362–1369.

Lee JS (2012): Albumin for end-stage liver disease. Korean Journal of Internal Medicine 27, 13–19.

Lehmann R, Koch M, Voelter W, Haring HU, Liebich HM (1997): Routine serum protein analysis by capillary zone electrophoresis: Clinical evaluation. Chromatographia 45, 390–396.

Lien TC, Wang JH (1995): Rapid differentiation of pathogenesis in patients with pulmonary edema by protein measurement with refractometer. Chinese Medical Journal (Taipei) 56, 298–304.

Lin AV (2015): Direct ELISA. Methods in Molecular Biology 1318, 61–67.

Lobetti RG, Mohr AJ, Dippenaar T, Myburgh E (2000): A preliminary study on the serum protein response in canine babesiosis. Journal of the South African Veterinary Association 71, 38–42.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951): Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193, 265–275.

Lumeij JT, de Bruijne JJ, Kwant MM (1990): Comparison of different methods of measuring protein and albumin in pigeon sera. Avian Pathology 19, 255–261.

Luraschi P, Dea E, Franzini C (2003): Capillary zone electrophoresis of serum proteins: effects of changes analytical conditions. Clinical Chemistry and Laboratory Medicine 41, 782–786.

Maciel RM, dos Anjos Lopes ST, Mazzanti CMA, Costa MM, Salbego FZ, Martins DB, Franca RT, Teixeira LV, Zanette RA (2012): A hematologic and electrophoretic study in puppies vaccinated against canine distemper virus and canine parvovirus. Acta Scientiae Veterinariae 40, 1011–1016.

Mackiewicz A (1997): Acute phase proteins and transformed cells. International Review of Cytology 170, 225–300.

Mair TS, Cripps PJ, Ricketts SW (1993): Diagnostic and prognostic value of serum protein electrophoresis in horses with chronic diarrhoea. Equine Veterinary Journal 25, 324–326.

Mallard BA, Dekkers JC, Ireland MJ, Leslie KE, Sharif S, Lacey-Vankampen C, Wagter L, Wilkie BN (1998): Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. Journal of Dairy Science 81, 585–595.

Mariani M, Luzzi E, Proietti D, Mancianti S, Casini D, Costantino P, van Gageldonk P, Berbers G (1998): A competitive enzyme-linked immunosorbent assay for measuring the levels of serum antibody to Haemophilus influenzae type b. Clinical and Diagnostic Laboratory Immunology 5, 667–674.

Matthews AG (1982): Serum protein electrophoresis in horses and ponies. Equine Veterinary Journal 14, 337–342.

McEwan AD, Fisher EW, Selman IE, Penhale WJ (1970): A turbidity test for the estimation of immune globulin levels in neonatal calf serum. Clinica Chimica Acta 27, 155–163.

McGrotty Y, Knottenbelt C (2002): Significance of plasma protein abnormalities in dogs and cats. In Practice 24, 512–517.

Medway W (1979): Bisalbuminemia in a bottlenosed dolphin (Tursiops truncatus). Experientia 35, 1158–1159.

Melmed GM (2009): Light chain amyloidosis: a case presentation and review. Proceedings (Baylor University. Medical Center) 22, 280–283.

Meyer DJ, Harvey JW (eds) (2004): Veterinary Laboratory Medicine: Interpretation and Diagnosis. 3rd edn. Saunders, Philadelphia. 156–168.

Morris D, Johnston JK (2002): Alterations in blood proteins. In: Smith BP (ed.): Large Animal Internal Medicine. 3rd edn. Mosby, St Louis. 426–433.

Nagy O, Tothova C, Nagyova V, Kovac G, Posivak J (2014): Changes in the serum protein electrophoretic pattern in lambs during the first month of life. Acta Veterinaria Brno 83, 187–193.

Nagy O, Tothova C, Nagyova V, Kovac G (2015): Comparison of serum protein electrophoretic pattern in cows and small ruminants. Acta Veterinaria Brno 84, 187–195.

O'Connell TX, Horite TJ, Kasravi B (2005): Understanding and interpreting serum protein electrophoresis. American Family Physician 71, 105–112.

Ochs HD, Smith CI (1996): X-linked agammaglobulinemia. A clinical and molecular analysis. Medicine (Baltimore) 75, 287.

Okutucu B, Dincer A, Habib O, Zihnioglu F (2007): Comparison of five methods for determination of total plasma

doi: 10.17221/19/2016-VETMED

protein concentration. Journal of Biochemical and Biophysical Methods 70, 709–711.

Orro T, Jacobsen S, LePage JP, Niewold T, Alasuutari S, Soveri T (2008): Temporal changes in serum concentrations of acute phase proteins in newborn dairy calves. Veterinary Journal 176, 182–187.

Pagana KD, Pagana TJ (eds) (2006): Mosby's Manual of Diagnostic and Laboratory Tests. 4th edn. Mosby Elsevier, St Louis. 1267 pp.

Paltrinieri S, Giordano A, Villani M, Manfrin M, Panzani S, Veronesi MC (2008): Influence of age and foaling on plasma protein electrophoresis and serum amyloid A and their possible role as markers of equine neonatal septicaemia. Veterinary Journal 176, 393–396.

Perille AL, Matus RE (1991): Canine ehrlichiosis in six dogs with persistently increased antibody titers. Journal of Veterinary Internal Medicine 5, 195–198.

Perryman LE, McGuire TC, Banks KL (1983): Animal model of human disease. Infantile X-linked agammaglobulinemia. Agammaglobulinemia in horses. American Journal of Pathology 111, 125–127.

Petersen HH, Nielsen JP, Heegaard PMH (2004): Application of acute phase protein measurements in veterinary clinical chemistry. Veterinary Research 35, 136–187.

Petersen JR, Okorodudu AO, Mohammad A, Payne DA (2003): Capillary electrophoresis and its application in the clinical laboratory. Clinica Chimica Acta 330, 1–30.

Perez JM, Gonzalez FJ, Granados JE, Perez MC, Fandos P, Soriguer RC, Serrano E (2003): Hematologic and biochemical reference intervals for Spanish ibex. Journal of Wildlife Diseases 39, 209–215.

Pfeiffer NE, McGuire TC (1977): A sodium sulfite-precipitation test for the assessment of colostral immunoglobulin transfer to calves. Journal of the American Veterinary Medical Association 170, 809–811.

Piccione G, Casella S, Giannetto C, Monteverde V (2009): Exercise-induced modifications on haematochemical and electrophoretic parameters during 1600 and 2000 meters trot races in standardbred horses. Journal of Applied Animal Research 35, 131–135.

Piccione G, Messina V, Schembari A, Casella S, Giannetto C, Alberghina D (2011): Pattern of serum protein fractions in dairy cows during different stages of gestation and lactation. Journal of Dairy Research 78, 421–425.

Piccione G, Messina V, Alberghina D, Giannetto C, Caselle S, Assenza A (2012a): Seasonal variations in serum protein fractions of dairy cows during different physiological phases. Comparative Clinical Pathology 21, 1439–1443.

Piccione G, Alberghina D, Marafioti S, Giannetto C, Casella S, Assanza A, Fazio F (2012b): Electrophoretic serum protein fraction profile during the different physiological phases in Comisana ewes. Reproduction in Domestic Animals 47, 591–595.

Pieper R, Gatlin CL, Makusky AJ, Russo PS, Schatz CR, Miller SS, Su Q, McGrath AM, Estock MA, Parmar PP, Zhao M, Huang ST, Zhou J, Wang F, Esquer-Blasco R, Anderson NL, Taylor J, Steiner S (2003): The human serum proteome: display of nearly 3700 chromatographically separated protein spots on two-dimensional electrophoresis gels and identification of 325 distinct proteins. Proteomics 3, 1345–1364.

Prinsen BH (2004): Albumin turnover: experimental approach and its application in health and renal diseases. Clinica Chimica Acta 347, 1–14.

Revillard JP (1980): Significance of beta 2-microglobulin in liver diseases. Vox Sanguinis 38, 339–342.

Riond B, Wenger-Riggenbach B, Hofmann-Lehmann R, Lutz H (2009): Serum protein concentrations from clinically healthy horses determined by agarose gel electrophoresis. Veterinary Clinical Pathology 38, 73–77.

Romdane MN, Romdhane SB, Jemli MH, Metoui K (1992): Electrophoretic profiles of serum proteins in dogs leishmaniasis (in French). Revue de Medecine Veterinarie 143, 753–755.

Rossi S, Bertazzolo W, Paltrinieri S, Giordano A (2008): Cellulose acetate electrophoresis of canine plasma after fibrinogen precipitation by ethanol. Veterinary Clinical Pathology 37, 422–428.

Roubies N, Panouis N, Fytianou A, Katsoulos PD, Giadinis H (2006): Effects of age and reproductive stage on certain serum biochemical parameters of Chios sheep under greek rearing conditions. Journal Veterinary Medicine A 53, 277–281.

Sapan CV, Lundblad RL, Price NC (1999): Colorimetric protein assay techniques. Biotechnology and Applied Biochemistry 29, 99–108.

Schrader M, Schulz-Knappe P (2001): Peptidomics technologies for human body fluids. Trends in Biotechnology 19, S55–S60.

Schroeder HW, Cavacini L (2010): Structure and function of immunoglobulins. Journal of Allergy and Clinical Immunology 125, S41–S52.

Sedlinska M, Krejci J, Vyskocil M (2005): Evaluation of field methods for determining immunoglobulins in sucking foals. Acta Veterinaria Brno 74, 51–58.

Sheldon IM, Noakes DE, Rycroft A, Dobson H (2001): Acute phase protein responses to uterine bacterial contamination in cattle after calving. Veterinary Record 148, 172–175.

Shetty JK, Prakash M, Gopalakarishna K (2007): Bisalbuminemia in adult male with Alzheimer's disease. Indian Journal of Medical Sciences 61, 356–357.

Slev PR, Williams BG, Harville TO, Ashwood ER, Bomhorst JA (2008): Efficacy of the detection of the alpha1-antitrypsin

doi: 10.17221/19/2016-VETMED

„Z" deficiency variant be routine serum protein electrophoresis. American Journal of Clinical Pathology 130, 568–572.

Spano JS, Whitesides JF, Pedersoli WM, Kristal LM (1988): Comparative albumin determinations in ducks, chickens, and turkeys by electrophoretic and dye-binding methods. American Journal of Veterinary Research 49, 325–326.

Stockham SL, Scott MA (2002): Fundamentals of veterinary clinical pathology. Iowa State Press, Ames. 251–276.

Swindle MM, Sistino JJ (2015): Anesthesia, analgesia, and perioperative care. In: Swindle MM, Smith AC (eds): Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques. 3rd edn. CRC Press, New York. 39–88.

Switzer R, Garrity L (eds) (1999): Experimental biochemistry. 3rd edn. WH Freemand and Company, New York. 451 pp.

Tappin SW, Taylor SS, Tasker S, Dodkin SJ, Papasouliotis K, Murphy KF (2011): Serum protein electrophoresis in 147 dogs. Veterinary Record 168, 456.

Tecles F, Spirane I, Bonfanti U, Ceron JJ, Paltrinieri S (2005): Preliminary studies of serum acute-phase protein concentrations in hematologic and neoplastic diseases of the dog. Journal of Veterinary Internal Medicine 19, 865–870.

Thomas LA, Brown SA (1992): Relationship between colloid osmotic pressure and plasma protein concentration in cattle, horses, dogs, and cats. American Journal of Veterinary Research 53, 2241–2244.

Tiselius A (1937): Electrophoresis of serum globulin II. Electrophoretic analysis of normal and immune sera. Biochemical Journal 31, 1464–1477.

Tothova C, Nagy O, Seidel H, Kovac G (2013a): Serum protein electrophoretic pattern in clinically healthy calves and cows determined by agarose gel electrophoresis. Comparative Clinical Pathology 22, 15–20.

Tothova C, Nagy O, Kovac G (2013b): The serum protein electrophoretic pattern and acute phase protein concentrations in calves with chronic respiratory diseases. Acta Veterinaria Beograd 63, 473–486.

Tothova C, Nagy O, Kovac G (2014): Acute phase proteins and their use in the diagnosis of diseases in ruminants: a review. Veterinarni Medicina 59, 163–180.

Tothova C, Nagy O, Kovac G, Nagyova V (2016a): Changes in the concentrations of serum proteins in calves during the first month of life. Journal of Applied Animal Research 44, 338–346.

Tothova C, Nagy O, Nagyova V, Kovac G (2016b): The concentrations of selected blood serum proteins in calves during the first three months of life. Acta Veterinaria Brno 85, 33–40.

Turba F, Enekel HJ (1950): Elektrophorese von Proteinen in Filterpapier. Naturwissenschaften 37, 93.

Tymchak LL (2010): Amino acids and proteins. In: Bishop ML, Fody EP, Schoeff LE (eds): Clinical Chemistry: Techniques, Principles, Correlations. 6th edn. Lippincott Williams and Wilkins, Philadelphia. 223–265.

Vavricka SR, Burri E, Beglinger C, Degen L, Manz M (2009): Serum protein electrophoresis: An underused but very useful test. Digestion 79, 203–210.

Vreugdenhil ACE, Dentener MA, Snoek AMP, Greve JWM, Buurman WA (1999): Lipopolysaccharide binding protein and serum amyloid A secretion by human intestinal epithelial cells during the acute phase response. Journal of Immunology 163, 2792–2798.

Watanabe A, Matsuzaki S, Moriwaki H, Suzuki K, Nishiguchi S (2004): Problems in serum albumin measurement and clinical significance of albumin microheterogeneity in cirrhotics. Nutrition 20, 351–357.

Weaver DM, Tyler JW, VanMetre DC, Hostetler DE, Barrington GM (2000): Passive transfer of colostral immunoglobulins in calves. Journal of Veterinary Internal Medicine 14, 569–577.

Webb J, Chary P, Northtrup N, Almy F (2008): Erythrophagocytic multiple myeloma in a cat. Veterinary Clinical Pathology 37, 302–307.

Weiss BM, Abadie J, Verma P, Howard RS, Kuehl WM (2009): A monoclonal gammopathy precedes multiple myeloma in most patients. Blood 113, 5418–5422.

Werner LL, Reavill DR (1999): The diagnostic utility of serum protein electrophoresis. Veterinary Clinics of North America: Exotic Animal Practice 2, 651–662.

Wilson K, Walker J (eds) (2000): Practical Biochemistry: Principles and Techniques. 5th edn. Cambridge University Press, Cambridge. 784 pp.

Woolf A, Nadler CF, Kradel DC (1973): Serum protein electrophoresis in bighorn sheep with chronic pneumonia. Journal of Wildlife Diseases 9, 7–11.

Wrotnowski C (1998): The future of plasma proteins. Genetic Engineering News 18, 14.

Yamada O, Tamura K, Yagihara H, Isotani M, Azakami M, Sawada S, Ono K, Washizu T, Bonkobara M (2007): Light-chain multiple myeloma in a cat. Journal of Veterinary Diagnostic Investigation 19, 443–447.

Yoshida Y (1991): Electrophoretic studies on serum proteins in cows with traumatic pericarditis. Journal of Veterinary Medical Science 53, 5–11.

Yuki M, Itoh H, Takase K (2010): Serum α-1-acid glycoprotein concentration in clinically healthy puppies and adult dogs and in dogs with various diseases. Veterinary Clinical Pathology 39, 65–71.

Zaia DAM, Zaia CTBV, Lichtig J (1998): Determination of total protein by spectrophotometry: advantages and disadvantages of proposed methods. Quimica Nova 21, 787–793.

Zaia DAM, Marques FR, Zaia CTB (2005): Spectrophotometric determination of total proteins in blood plasma:

doi: 10.17221/19/2016-VETMED

a comparative study among dye-binding methods. Brazilian Archives of Biology and Technology 48, 385–388.

Zini E, Bonfanti U, Zatelli A (2004): Diagnostic relevance of qualitative proteinuria evaluated by use of sodium dodecyl sulphate-agarose renal histologic findings in dogs. American Journal of Veterinary Research 65, 964–971.

Zor T, Selinger Z (1996): Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies. Analytical Biochemistry 236, 302–308.

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