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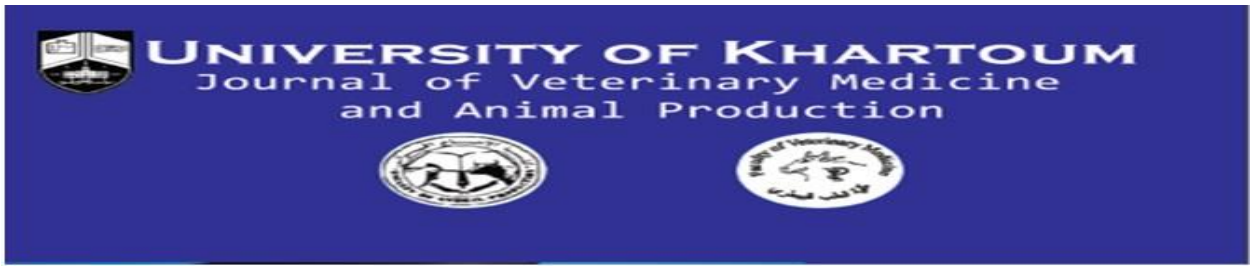

Influence of Age on Serum Protein Capillary Electrophoretic Pattern in Clinically Healthy Calves with Special Emphasis on Serum Globulins

Nawal M. Elkhair[1*] and H. Hartmann[2]

Abstract

The objective of this study was to characterise serum protein capillary electrophoretic pattern in relation to the age in calves. Thirty two German Black-coloured and crossbred calves (age: 4-104 days) were used. Venous blood samples were collected from the jugular vein and were used for the determination of serum protein; by capillary electrophoresis technique. The age of the calves correlated positively ($P<0.01$) with serum albumin concentration and albumin/globulin ratio. The youngest calves in the first week of life showed significantly ($P<0.05$) lower mean serum albumin compared to the older calves of 2-3 months. The concentrations of α_1, β- and γ- globulin were not affected significantly by age. The young calves in the 2nd week of life showed significantly ($P<0.01$) higher mean value for α_2 globulin concentration compared to the old calves (1-3 months). Albumin/globulin ratio showed significantly ($P<0.01$) higher value in the young calves in the 2nd week of life compared to the old calves in the age of 1-2 months. These values were compared to those reported by other investigators in calves, adult cattle and other animals. The results indicate that the serum electrophoresis pattern of the calves is influenced by age.

Key words: calves, age, serum protein, capillary electrophoresis.

المستخلص

تمت دراسة نمط الترحيل الكهربي الشعيري لبروتينات مصل الدم وعلاقته بالعمر. أجريت الدراسة في 32 عجل من السلالة الألمانية ذات اللون الأسود والسلالة الهجين (العمر: 4-104 يوما). جمعت عينات من الدم الوريدى من الوريد الوداجى وإستخدمت لتقدير بروتينات مصل الدم بإستخدام تقنية الترحيل الكهربي الشعيري. أوضحت النتائج أن عمر العجول إرتبط إرتباطا إيجابيا ($P< 0.01$) مع تركيز ألبومين المصل ونسبة الألبومين/القلوبيولين. أظهرت العجول الأصغر سناً في الأسبوع الأول من الحياة إنخفاضاً معنويا ($P< 0.01$) فى متوسط ألبومين مصل الدم بالمقارنة مع العجول كبيرة السن فى عمر 2-3 أشهر. ألفا1(α_1) ، جاما (γ) وبيتا (β)- قلوبيولين لم تتأثر بشكل ملحوظ بالعمر. أظهرت العجول الصغيرة في الاسبوع الثانى من الحياة أعلى قيمة ذات دلالة إحصائية ($P< 0.01$) لتركيز القلوبيولين ألفا2 (α_2) بالمقارنة مع العجول كبيرة السن (1-3 أشهر). أظهرت نسبة الألبومين/القلوبيولين أعلى قيمة معنوية ($P< 0.01$) في العجول الصغيرة في الاسبوع الثانى من الحياة بالمقارنة مع العجول كبيرة السن في عمر 1-2 أشهر. تمت مقارنة هذه القيم مع تلك التى تم تسجليها

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من قبل باحثين آخرين في الأبقار البالغة، الإنسان وغيرها من الحيوانات. أشارت النتائج أن للعمر تأثيراً معنوياً على نمط الترحيل الكهربي الشعيري لبروتينات مصل دم العجول.

Introduction

Serum proteins are known to comprise about 6-7 g/dl of the plasma (Eckersall, 2008). Functionally, plasma proteins are involved in nutrition, maintenance of osmotic pressure, buffering acid-base balance, transport of smaller ions and molecules, haemostasis and protective effect of the immune proteins (Eckersall, 2008). Albumin is the most osmotically active serum protein and it is also an important carrier of many substances. Globulins are a heterogeneous group of proteins that include antibodies and other inflammatory molecules, haemostatic and fibrinolytic proteins, and carriers of lipids, vitamins, and hormones (Eckersall, 2008). Many of the plasma proteins change markedly in diseases (Abate *et al.*, 2000; Rasouli *et al.*, 2005) and with age (Keay and Doxy, 1982; Chen *et al.*, 1999; Chaudhary *et al.*, 2003; Piccione *et al.*, 2009; Tothova *et al.*, 2014).

Capillary electrophoresis of serum proteins (CE) is an established and effective method which has been used as a screening tool for the clinical diagnosis of many diseases in humans (Jellum *et al.*, 1997; Gay-Bellile *et al.*, 2003) and animals (Camacho *et al.*, 2005; Facchini *et al.*, 2010). In clinical practice, CE has been developed and applied to the simultaneous analysis of a great variety of substances of clinical importance such as proteins (Giordano and Paltrinieri, 2010), organic acids (Jager *et al.*, 2003) as well as urine (Jellum *et al.*, 1997), cerebrospinal fluid and hair (Petersen *et al.*, 2003). Recently, many researchers have demonstrated the use of CE in different animal species (Crivellente *et al.*, 2008; Giordano and Paltrinieri, 2010; Piccione *et al.*, 2011; Tothova *et al.*, 2013; Elkhair and Hartmann, 2014) and in a variety of protein abnormalities (Facchini *et al.*, 2010; Giordano and Paltrinieri, 2010).

Normal serum proteins capillary celectrophoretic pattern (CEP) is composed of five fractions, albumin, α_1-globulin, α_2 globulin, β-globulin and γ- globulin (Eckersall, 2008). Therefore, the clinical interpretation of CEP is based on the variation in the content of one or more of these five major fractions. However, species differences between the animals have been observed by Keay and Doxy (1982). Therefore, the aim of the study was to validate the use of CE in calves and to determine the influence of age in the normal serum protein capillary electrophoretic pattern.

Materials and Methods

Animals: A total number of 32 clinically healthy calves (German Black-coloured and crossbred) were used. The calves were assigned to 6 groups according to their age, i.e. 1st week: n=3, 2nd week: n=6, 3rdweek: n=5, 4thweek: n=4, 1-2 month(s): n=9 and 2-3 months: n=5. The calves were housed individually in stalls in indoor pens and standard calf management procedures were used. Milk was provided three times daily (7:30 am, 13:30 and 22:30 pm) and the calves had free access to fresh water. Hay and grain were offered when the calves were 2 weeks old.

Sample collection and laboratory analysis: Venous Blood samples were collected from the jugular vein using plastic syringes (7.5 ml, Pirmvetta®, Laboratory Technique, GmbH, Germany). The samples were centrifuged and the serum was collected in sterile containers for analysis. The fractionation of serum proteins was determined using a capillary electrophoresis technique using a biochemical analyser (Roche Hitachi Modular, Roche).

Statistical analysis was performed using SPSS for Windows version 17.0. The statistical measurements of serum total protein fraction were estimated using descriptive statistics procedures of the same programme. ANOVA tests (Levine's Test and Post Hoc Test) were used to assess the possible significant differences between the age groups. The mean difference was considered significant at $P \leq 0.05$.

Results

Fig.1 shows the normal pattern of CE in healthy calves of various ages. The pattern of CE identified one albumin, two α- globulin (α_1 and α_2), one β-globulin and one γ-globulin fractions for the age group of 4th week and the older calves of 2-3 months. However, the young calves (1-3 week) and the old calves showed two β-globulin fractions (β_1 and β_2).

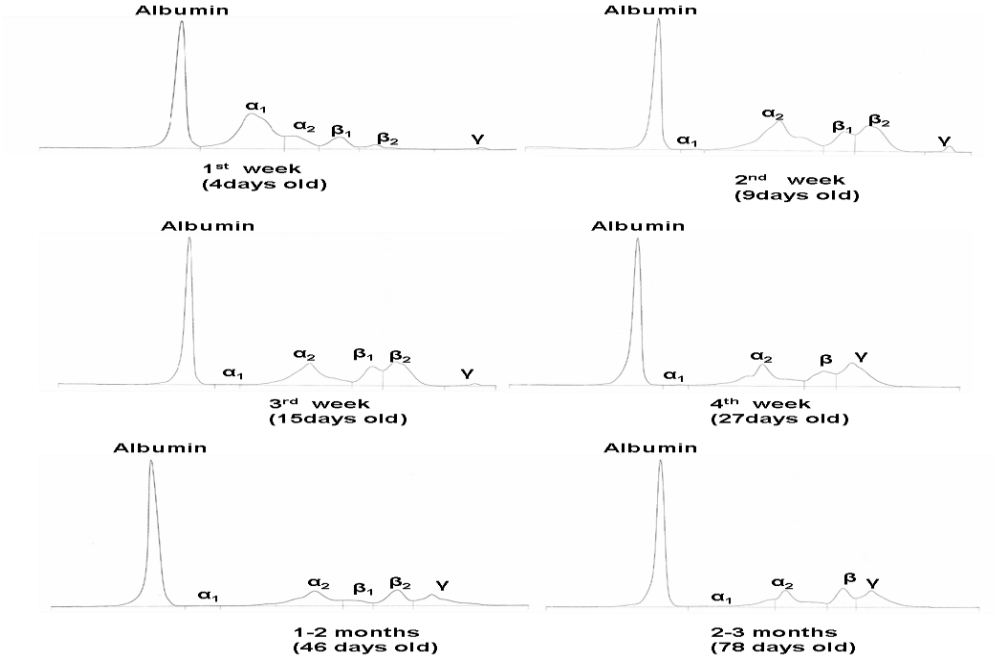


Fig. 1 Serum protein capillary electrophoresis pattern of healthy calves of various ages (n = 32).

Table 1 shows that the age of the calves correlated positively ($P<0.01$) to the serum albumin concentration and the ratio of albumin/globulin. Table 2 summarised the effect of age on serum total protein, albumin, and globulin concentrations and albumin/globulin ratio. The detailed results presented in Fig. 2 A and B indicate that the mean values of serum total protein fluctuated with age. However this pattern of response was not statistically significant. Serum albumin concentration increased gradually with advanced age. The younger calves in the 1st week of life showed a significant ($P<0.05$) lower mean of serum albumin compared to the older calves of 2-3 months. Also the young calves in the 2nd week of life showed significantly ($P<0.01$) lower values of serum albumin compared to the calves in the 4th week of life and the old calves in the age of 1-3 months.

Fig. 3A and B indicate that α_1- globulin was not affected significantly by the age, the young calves in the 2nd week of life had significantly ($P<0.01$) higher mean value for α_2 globulin concentration compared to the old calves (1-3 months). The values of β- and γ- globulins were not affected significantly by the age (Fig. 4A, B and Fig. 5A, respectively). Albumin/globulin ratio (A/G) increased gradually with age and significantly ($P<0.01$) higher ratio was observed in the young calves in the 2nd week of life compared to aged 1-2 months.

Table 1 Correlation between the age and serum total protein, albumin and globulin concentrations (g/l) and albumin/globulin ratio in healthy calves (n=32)

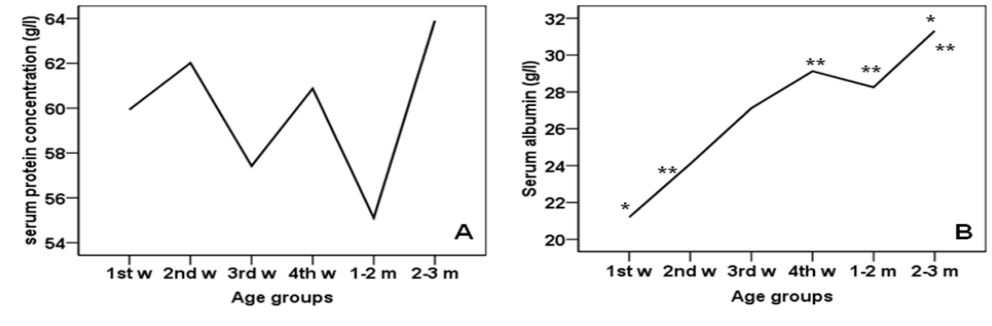
Parameter	Pearson correlation	Level of Significance
Age - serum total protein (g/l)	0.14	0.44
Age - serum albumin (g/l)	0.75 *	0.0001
Age - alpha 1 globulin (g/l)	-0.09	0.66
Age - alpha 2 globulin (g/l)	-0.34	0.07
Age - beta 1 globulin (g/l)	0.08	0.67
Age - beta 2 globulin (g/l)	-0.28	0.5
Age - gamma globulin (g/l)	0.29	0.13
Age - albumin/globulin ratio (A/G)	0.5 *	0.01

* Correlation is significant at the 0.01 level.

Table 2 Effect of age on serum total protein, albumin and globulin concentrations(g/l) albumin/globulin ratio in healthy calves (n=32)

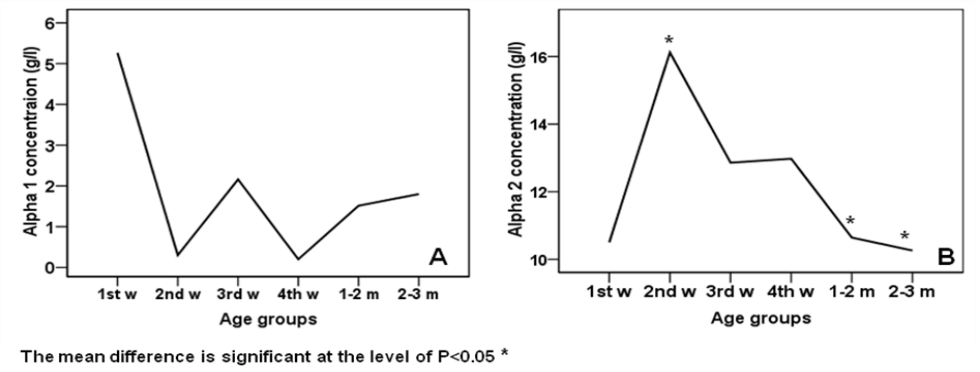
Parameter	Age					
	weeks				months	
	1st	2nd	3rd	4th	1-2	2-3
Number of calves	3	6	5	4	9	5
Serum total protein (g/l)	60 a ±16	62 a ±7	57a ±4	61 a ±5	55 a ±4	64 a ±5
Serum albumin (g/l)	21 a ±2.6	24 a ±1.2	27 ab ±1.3	29 ab ±1.4	28 ab ±1.9	31 b ±2.2
Globulin (g/l)	39 a ±14.7	38 a ±6.6	30 a ±9.8	32 a ±6	27 a ±3.7	33 a ±6.8
Alpha 1 globulin (g/l)	5.3 a ±0.5	0.3 a ±0.1	2.2 a ±0.1	0.2 a ±0.1	1.5 a ±0.1	1.8 a ±0.01
Alpha 2 globulin (g/l)	10.5 ab ±6	16 b ±2	12.9 ab ±6	13 ab ±1.7	10.6 a ±2	10 a ±2
Beta 1 globulin (g/l)	4.9 a ±1.8	6.6 a ±1	6.3 a ±0.5	6.6 a ±0.9	5.9 a ±3.4	6.5 a ±1.4
Beta 2 globulin (g/l)	11.5 a ±1.9	10.1 a ±2.1	10.9 a ±4	a 5.7 ±0.4	No β_2 fraction	No β_2 fraction
Gamma globulin (g/l)	6.9 a ±1.2	10 a ±9	8 a ±5	12 a ±4.8	8.7 a ±2.3	13.8 a ±6
Albumin/globulin ratio (A/G)	0.6 ab ±0.2	0.7 a ±0.1	0.9 ab ±0.1	0.9 ab ±0.2	1.1 b ±0.1	1 ab ±0.2

Means within the same row bearing different superscripts are significantly different at P ≤0.05



The mean difference is significant at the level of P<0.05 *, P<0.01 **

Fig. 2 A, B Mean values of serum total protein and albumin concentrations (g/l) in calves (n=32)



The mean difference is significant at the level of P<0.05 *

Fig. 3 A, B Mean values of alpha 1 and 2 globulins concentration (g/l) in calves (n=32)

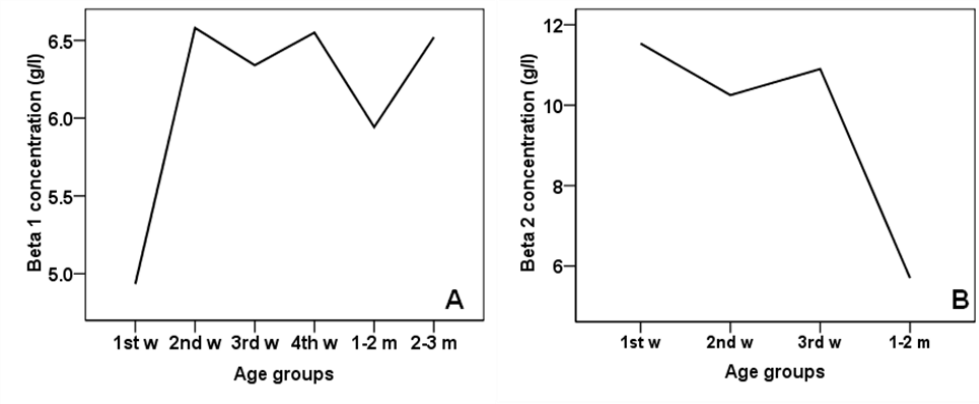
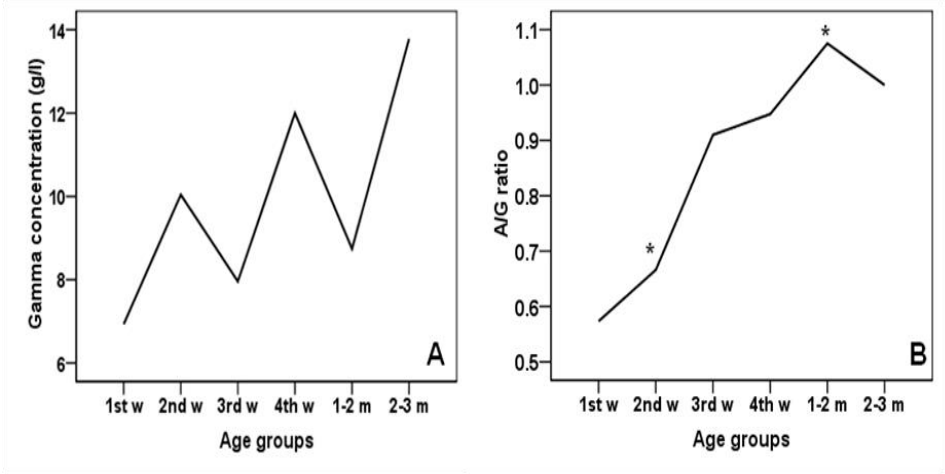


Fig. 4 A, B Mean values of beta 1 and 2 globulins concentration (g/l) in calves (n=32)



The mean difference is significant at the level of P<0.05 *

Fig. 5 A, B Mean values of gamma globulin concentration and albumin/globulin ratio (A/G) (g/l) in calves (n=32)

Discussion

The main finding of the present study is that CE has been applied to the serum of the calves. CE produced five peaks comprising one albumin, α_1 and α_2, β and γ- globulins fractions (Fig. 1). However, Tothova et al., (2014) have reported that serum protein electrophoresis on agarose gel, produced six peaks comprising one albumin, α_1 and α_2, β_1 and β_2 and γ- globulin fractions in calves. The variation in the appearance of protein fractions may be attributed to the method used. Albumin represented the main fraction of serum proteins determined by CE in all age groups (Fig 1). In the 1st week of life, α_1 represented higher fraction compared with α_2, β and γ- globulin fractions. However, with advanced age α_1 represented the lowest fraction compared to the other globulins. From the 4th week of life to 2-3 months, γ- globulin showed higher fraction compared to the other globulins. The higher concentrations of γ-globulins in older calves compared to the values in younger animals may be attributed to good alimentary canal absorption and the normal process of growth or due to the gradual increase in immunoglobulins (Paltrinieri *et al.,* 2008). Similar changes were observed in calves by other investigators (Mohri *et al.,* 2007; Tothova *et al.,* 2014). Furthermore, Chaudhary *et al.* (2003) and Elkhair and Hartmann (2014) suggested that lower concentrations of γ-globulins in young calves are caused by the immaturity of the lymphoid system or immature immune system which remain low until the production of globulin by the maturing immune system. The variation in these values can be considered as age-dependent relationship between the groups. However, the young calves until the 3rd week of life showed β_1 and β_2 globulins fraction accompanied by lower γ- globulins fraction compared to the other age groups. Previously, Keay and Doxey (1982) found higher values of α- and β-globulins between calves at the age of 3-4 weeks and adult cattle..

The mean values of serum total protein concentration obtained in the present study for calves (55-64 g/l, Fig 2B) were similar to the reference range reported by Stöber and Gründer, (1990). In the older calves, the higher mean value of serum protein concentration (64 g/l) can be attributed to the higher concentration of γ- globulins observed (26%=16.8 g/l) (Fig. 5A).

It has been reported previously that the age may markedly influence the electrophoretic pattern of serum proteins with decreasing albumin and increasing globulin concentrations with advancing age in calves, goats, and camels (Tothova *et al.*, 2014; Alberghina *et al.,* 2010; Elkhair and Hartmann, 2014). In the present study the initial mean values of albumin concentration for the youngest calves in the 1st week of life are lower compared with the reference range reported by Stöber and Gründer (1990). This may be attributed to the increase in serum globulin concentration observed, particularly β_2 concentration (Fig. 4B). These results are in agreement with those reported previously by Knowles *et al.* (2000).

The present results indicate that the increased albumin/globulin ratio (A/G) may be attributed to decreased albumin and increased globulin concentrations with advancing age. Kaneko (1997) reported that albumin/globulin ratio is of special interest for clinicians because it allows systematic classification of the electrophoretic profile and identification of dysprteinaemias. Furthermore, Alberghina *et al.* (2010) recommended that the A/G ratio should be interpreted cautiously with special emphasis on their change.

Conclusion

The present study indicates that the serum electrophoresis pattern of the calves is influenced by age in addition to genetic and the nutritional status of the animals. The data could be utilised for clinical monitoring of dysprteinaemias and to establish reference intervals for capillary electrophoresis in healthy calves.

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