Acta vet. scand. 1980, 21, 482-497.

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CONCENTRATION OF SERUM PREALBUMIN (PR) PROTEIN IN SICK HORSES AND ITS CORRELATION TO BLOOD LEUCOCYTE COUNT AND ALBUMIN CONTENT IN SERUM

By

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EK, NILS: Concentration of serum prealbumin (Pr) protein in sick horses and its correlation to blood leucocyte count and albumin content in serum. Acta vet. scand. 1980, 21, 482-497. — Studies of Pr protein concentrations in sera of sick horses were carried out using Mancini et al.'s (1965) immunodiffusion technique. Relative values against a chosen standard of 100 % were determined for a total of 102 horses. Horses with acute infections had Pr protein values signifi-cantly above the normal. The highest individual Pr protein value recorded in this group was 202. Horses suffering from acute laminitis and malignant tumours also had increased Pr protein values. There was a positive correlation between the Pr protein value and the blood leucocyte count and a negative correlation between the Pr protein value and the albumin content in serum.

horse; serum; prealbumin (Pr) protein concentration.

Quantification of the Pr protein in horse sera revealed significant differences between Pr phenotypes (Ek & Brænd 1980). The average Pr protein concentration of Pr SS and Pr UU phenotypes were lower than those of horses not having the Pr S and Pr U alleles, the Pr UU phenotypes being significantly the lowest. Furthermore the average Pr protein concentration was significantly higher in foals than in mares.

In man, individuals homozygous for the Pr Z allele show an α_1 -antitrypsin deficiency and often suffer from lung emphysema (Ganrot et al. 1967), an explanation being that α_1 -antitrypsin inhibits the proteases in human granulocytes which are active during inflammatory processes (Ohlson 1971).

Corbella (1975) carried out preliminary investigations on serum antiproteases in sick horses and reported variations between horses suffering from diseases of the respiratory, digestive, locomotor and genital systems.

Breeze et al. (1977) measured serum antitrypsin activity in horses with chronic pulmonary disease, but found no significant differences between these and animals without respiratory diseases.

The purpose of the work reported in the present paper was to investigate the possible effect of diseases on Pr protein concentration and, in addition, to correlate the Pr protein value with blood leucocyte count and serum albumin content.

MATERIALS AND METHODS

The investigations were carried out on 102 horses, hospitalized at the Department of Internal Medicine I, Veterinary College of Norway. The horse material included 95 horses aged two to 20 years, comprising 45 Warmblood Trotters, 38 Norwegian Trotters, six mixed breeds, three Norwegian Fjord horses, one Nordland horse, one Iceland horse and one Shetland pony. In addition there were five Warmblood Trotter foals and two Norwegian Trotter foals aged 10 days to 12 months. The material was divided into seven groups as will be seen under Results.

The horses in the disease groups were subdivided according to their Pr phenotypes. Pr protein values of homozygotes and heterozygotes for Pr U and Pr S were tested separately. Foals were separated from adult horses.

The horses were bled when they arrived at the clinic. Serum was pipetted off and stored at -20°C when not used immediately. Four horses were repeatedly tested during the hospitalization period.

Determination of Pr phenotypes was performed according to the technique of Brænd (1970). Quantification on the Pr protein was carried out as previously described (*Ek & Brænd* 1980), using the method of *Mancini et al.* (1965). The serum from a Pr FF horse, chosen as a standard, was the same as in an earlier work (*Ek & Brænd*). The quantity of the Pr protein of this horse was given the value of 100 %.

All quantification tests were carried out in duplicate. The standard deviation of the double tests was calculated by the

formula $s = \sqrt{\frac{\Sigma \cdot d^2}{2 n}}$, where d is the difference between the

double tests and n is the number of double tests. The standard deviation in the material was 1.6.

The albumin percentage in serum was determined by electrophoresis on cellulose acetate membrane by the method described by Ek (1970), and the total protein determined according to the method of *Dimopoullos* (1970), whereafter the albumin content was calculated.

The blood for leucocyte count was taken from the jugular vein with pulverized EDTA as an anticoagulant, 20 mg to 10 ml blood. The leucocyte count was performed with a Celloscope 401 AB Lars Ljungberg & Co according to the Instruction Manual.

Standard methods were employed for the statistical calculations (Snedecor & Cochran 1967).

RESULTS

Group I. Twenty-six horses with acute infections

The horses in this group were patients with a history of disease running a rapid course and with clinical signs of an acute infection (fever, pain and functional disturbance of the diseased organ). Post-mortem examination was carried out in four horses. Table 1 shows that the Pr protein values in all Pr phenotype classes investigated were increased as compared with the figures previously found in healthy horses (*Ek & Brænd* 1980). The highest mean value was found in Pr phenotypes without U and S alleles. The Pr protein value in this group was significantly higher than the corresponding value found in healthy horses. A significant difference was also found in Pr S heterozygotes.

The mean albumin values were below the normal range given by *Dimopoullos* (1970) in all Pr phenotype classes. The mean values of the leucocyte count were above the normal range given by *Schalm* (1967). The patient having the highest Pr protein value in the whole material was a 12-year-old gelding Norwegian Trotter with a history of constant fever for a period of seven days before admittance. The horse belonged to the National Veterinary Institute of Norway and had been used in the production of antiserum against Escherichia coli. The clinical signs on admittance were a temperature of 38.6°C, weakness and poor appetite. The horse had a Pr protein value of 202 % (phenotype

Adult	Pr %	Albumin g/100 ml	Leucocytes per mm ³ ×10 ³
Pr II hom	ozvgotes		· · · · · · · · · · · · · · · · · · ·
Number	1	1	1
Number	104.0	28	29.5
Normal	50 092 5	2.8-3.5	6 0-12 0
	00.0-02.0	2.0 0.0	0.010.0
Pr U hete	rozygotes		
Number	3	3	3
Range	95143	2.3 - 3.0	7.7-20.2
Mean	112.7	2.7	14.3
Normal	61.8-88.8	2.8-3.5	6.0-12.0
Pr S hete	rozygotes		
Number	4	4	4
Range	100135	2.2 - 3.1	8.1-25.8
Mean	124.3	2.7	13.8
Normal	57.7-102.7	2.8-3.5	6.0-12.0
	P < 0.001		
(Co	mpared with 17 healt	hy mares)	
Pr phenot	vpes without L	Jand S	
Number	13	13	10
Range	98202	1.5-3.8	6.2 - 20.3
Mean	131.5	2.7	10.9
Normal	63.7-103.7	2.8 - 3.5	6.0-12.0
	P < 0.001		
(C o	mpared with 23 healt	hy mares)	
Foals			
Pr U hete	rozygotes		
Number	2	2	2
Range	118150	2.2 - 2.5	11.9-20.4
Mean	134.0	2.35	16.9
Normal	62.4-90.4	2.8-3.5	7.6—18.6
Pr S hete	rozygotes		
Number	2	2	2
Range	83-125	1.3-2.4	11.4-26.5
Mean	104.0	1.85	19.0
Normal	64.5—111.5	2.83.5	7.6-18.6
Pr phenot	ypes without l	Jand S	
Number	1	1	1
	118.0	1.5	16.3
Normal	72.3-122.6	2.8 - 3.5	7.6-18.6
	••		

Table 1. Concentration of serum Pr protein, serum albumin and blood leucocyte count in horses with acute infections (Group I). Corresponding values for the other groups are given in Tables 2-7.

NN), albumin 2.1 g per 100 ml and leucocytes 17700/mm³. The horse died after five days.

Post-mortem report: A large abscess was found in the liver containing 10 l of thick yellow pus. The liver was enlarged and there were massive adhesions between the spleen and diaphragm and between the spleen and the peritoneum. Section from liver and spleen were positive when stained for amyloidosis.

The highest Pr protein value among the foals was found in a male Warmblood Trotter foal aged 10 days. The foal was presented with a history of severe lameness and difficulty in getting onto its feet.

The clinical signs were a temperature of 40° C and recumbency most of the time. There was obvious swelling and tenderness of the left elbow and the right hock joints. Puncture of the elbow produced a thick yellow synovia which coagulated shortly afterwards. No microorganisms could be isolated. The foal had a Pr protein value of 150 % (phenotype LU), albumin 2.2 g per 100 ml and leucocytes 20400/mm³.

The foal was treated daily with antibiotics and made steady improvement. It was discharged after three weeks as clinically improved. The Pr protein value at that time had decreased to 87 %, which is within the normal range.

Fig. 1 shows the Pr protein concentration in sera from four horses with acute infections tested repeatedly during the hospitalization period. One of the horses was destroyed after six days in a moribund condition. In this case the Pr protein concentration increased during the hospitalization period reaching a maximum at death. The three other horses recovered. In one of these, however, the Pr protein value reached a maximum four days after admittance, while in the two remaining animals the Pr protein value decreased rapidly after having been evelated on arrival.

Group II. Seven horses with acute laminitis

The animals in this group were horses with a history of an acute disease and clinical signs characteristic of acute laminitis such as severe pain in the feet, rapid pulse and respiration and reluctance to move. No post-mortem examinations were performed in this group.

Table 2 shows that the mean Pr protein values in this disease group were increased as compared with corresponding values



Figure 1. Pr protein concentration in sera from four horses with acute infections tested repeatedly during the hospitalization period. Horse 99/79 was destroyed after six days in moribund condition whereas the others recovered.

in healthy horses. In Pr phenotypes without U and S alleles the increase was significant. The mean values of albumin were decreased.

The mean leucocyte counts were within the normal range.

Group III. Twenty-four horses with chronic obstructive pulmonary disease (COPD)

This group included horses with confirmed chronic disease of the lungs. These animals exhibited a variety of clinical respiratory abnormalities resulting in a chronic pulmonary dysfunction.

The diagnosis was made on the basis of clinical examination and case history. The horses were not examined at post-mortem.

The mean values for Pr protein in all Pr phenotype classes were within the normal range for horses with corresponding Pr phenotypes (Table 3). Furthermore the mean values appeared

Adult	Pr %	Albumin g/100 ml	Leucocytes per mm ³ ×10 ³
Pr S hom	ozvgotes		
Number	1	1	1
	109.0	2.6	7.5
Normal	58.2-94.6	2.8 - 3.5	6.0-12.0
Pr S hete	rozygotes		
Number	3	3	3
Range	106-114	2.2 - 2.9	7.5-12.7
Mean	109.6	2.6	9.2
Normal	57.7-102.7	2.8-3.5	6.0-12.0
Pr phenot	ypes without U	and S	
Number	4	4	4
Range	123	2.5 - 2.8	6.9-13.4
Mean	127.0	2.6	8.8
Normal	63.7-103.7	2.8 - 3.5	6.0-12.0
	P < 0.001		
(Co	mpared with 23 healt	hy mares)	

Table 2. Horses with acute laminitis (Group II).

Table 3. Horses with chronic obstructive pulmonary disease (Group III).

Adult	Pr %	Albumin g/100 ml	Leucocytes per mm ³ ×10 ³
Pr U home	ozvgotes		
Number	3	3	3
Range	6887	2.8-3.6	6.4-8.7
Mean	75.0	3.2	7.7
Normal	50.5-92.5	2.8-3.5	6.0-12.0
Pr U hete	rozygotes		
Number	1	1	1
	96.0	2.8	15.3
Normal	61.8—88.8	2.8-3.5	6.0-12.0
Pr S hete	rozygotes		
Number	8	8	8
Range	61—96	2.8 - 3.5	6.8-10.8
Mean	84.1	3.3	8.4
Normal	57.7-102.7	2.8 - 3.5	6.0-12.0
Pr phenot	ypes without U	and S	
Number	12	10	11
Range	75—111	3.13.8	6.7-9.8
Mean	87.7	3.4	8.2
Normal	63.7—103.7	2.8 - 3.5	6.0-12.0

to be very close to mean values found in healthy horses. The albumin and the leucocyte count were all within the normal range.

Group IV. Nine horses with chronic enteritis

The horses in this group were animals exhibiting signs of enteritis (diarrhoea) which had lasted for more than one week. No post-mortem examinations were performed.

All mean values (Table 4) were within the normal range, though the mean Pr protein value for phenotypes without U and S alleles was close to the lower limit of the normal range.

Adult	Pr %	Albumin g/100 ml	Leucocytes per mm³×10³
Pr U home	ozygotes		
Number	2	2	2
Range	68—86	3.43.6	6.9-7.8
Mean	77.0	3.5	7.4
Normal	50.592.5	2.8 - 3.5	6.0-12.0
Pr S hete	rozygotes		
Number	2	2	2
Range	79—110	2.0 - 3.4	7.1-8.3
Mean	94.5	2.7	7.7
Normal	57.7-102.7	2.8 - 3.5	6.0-12.0
Pr phenot	ypes without U	and S	
Number	3	3	3
Range	50-81.5	2.8-3.2	5.7-10.7
Mean	69.5	3.0	8.0
Normal	63.7—103.7	2.8-3.5	6.0-12.0
Foals			
Pr U hete	rozygotes		
Number	1	1	1
	85.0	3.5	15.8
Normal	62.4—90.4	2.8 - 3.5	7.6—18.6
Pr phenot	ypes without U	and S	
Number	1	1	1
	100.0	3.0	12.0
Normal	72.3-122.6	2.8-3.5	7.6—18.6

Table 4. Horses with chronic enteritis (Group IV).

Group V. Four horses with malignant tumours

This group consisted of horses suffering from different malignant tumours. Diagnosis was based on clinical signs and postmortem examination.

The results for this group are presented in Table 5. The mean values of the Pr protein in both Pr phenotype classes were considerably above Pr protein values in healthy horses. Albumin values were below normal. The leucocyte count was increased in Pr S-heterozygotes but within the normal range in Pr U-heterozygotes.

Adult	Pr %	Albumin g/100 ml	Leucocytes per mm³×10³
Pr U hete	rozygotes		
Number	2	2	2
Range	90-157	1.9-2.2	7.8-10.1
Mean	123.5	2.05	9.0
Normal	61.8-88.8	2.8 - 3.5	6.012.0
Pr S hete	rozygotes		
Number	2	2	2
Range	106—141	2.2 - 2.5	13.5-44.7
Mean	123.5	2.35	29.1
Normal	57.7-102.7	2.8 - 3.5	6.0-12.0

Table 5. Horses with malignant tumours (Group V).

The highest Pr protein value in this disease group was found in a Warmblood Trotter mare aged 14 years. She had been sick for five weeks with weight loss and distended abdomen. The clinical signs were depression, temperature of 37.2° C, diarrhoea and anorexia. The horse had a Pr protein value of 157 % (phenotype LU). The albumin value was 1.9 g per/100 ml and the leucocyte count 10100/mm³. One day after arrival at the clinic, the patient was unable to rise and was therefore destroyed.

Necropsy report: An adenocarcinoma weighing 23 kg was found in the abdomen. It had infiltrated the ventricle.

Another horse in the tumour group was a Norwegian Trotter gelding, 15 years old, with a case history of fever and weight loss.

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Clinical signs: A temperature of 40° C, depression and anorexia. The horse had a Pr protein value of 141 % (phenotype ST). The total leucocyte count was $44700/\text{mm}^{\circ}$, with 93 % lymphocytes.

The most significant laboratory finding was the existence of a severe hypergamma-globulinaemia characterized by a monoclonal spike of gammaglobulin (5.0 g per 100 ml), whereas the albumin was 2.1 g per 100 ml.

Due to its serious condition the horse was destroyed after seven days. Post-mortem: Lymphosarcoma infiltrating the liver, the kidneys, the myocardium and the lymph nodes.

Group VI. Seven horses with colic

This group incorporated horses exhibiting abdominal pain associated with various diseases of the abdominal viscera such as intestinal impaction and intestinal tympani. Post-mortem examination was carried out in three horses.

All the Pr protein values in this group were within normal range with the exception of one horse having an increased value (Table 6). The mean values for albumin and leucocyte count were normal.

Adult	Pr %	Albumin g/100 ml	Leucocytes per mm ³ ×10 ³
Pr U hete	rozygotes		
Number	1	1	1
	96.0	3.1	8.1
Normal	61.888.8	2.83.5	6.0-12.0
Pr S homo	zygotes		
Number	1	1	1
	85.0	3.8	9.9
Normal	58.2-94.6	2.83.5	6.0-12.0
Pr phenot	ypes without U	Jand S	
Number	5	5	4
Range	83104	3.13.9	7.3-9.8
Mean	94.4	3.4	8.4
Normal	63.7-103.7	2.8 - 3.5	6.012.0

Table 6. Horses with colic (Group VI).

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Group VII. Twenty-five horses with organic diseases.

This group comprised horses which were suffering from different organic conditions not associated with inflammatory reactions (myopathy, arthrosis, eczema etc.). No post-mortem examinations were carried out. Results are shown in Table 7. All the mean values were normal.

Adult	Pr %	Albumin g/100 ml	Leucocytes per mm ³ ×10 ³
	/0	B /100 IIII	per mm X10-
Pr U hom	ozygotes		
Number	3	3	3
Range	68—100	3.2 - 3.5	6.4-11.5
Mean	83.3	3.3	9.9
Normal	50.0-92.5	2.8-3.5	6.0-12.0
Pr U hete	rozygotes		
Number	5	5	5
Range	64—89	2.1 - 3.7	7.69.8
Mean	79.8	3.0	8.9
Normal	61.8-88.8	2.8-3.5	6.0-12.0
Pr S hete	rozygotes		
Number	9	7	8
Range	5494	2.5 - 3.4	7.6 - 12.4
Mean	75.4	3.1	9.4
Normal	57.7—102.7	2.8-3.5	6.0—12.0
Pr phenot	ypes without U	Jand S	
Number	8	8	8
Range	70—109	2.8 - 3.5	4.812.4
Mean	88.9	3.1	8.6
Normal	63.7-103.7	2.8 - 3.5	6.0-12.0

Table 7. Horses with organic diseases (Group VII).

Figure 2 shows a scatter diagram of Pr protein concentration in relation to leucocyte count in 37 horses (Pr phenotypes without U and S alleles) in the present material.

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F i g u r e 2. A scatter diagram of Pr protein values in serum in relation to leucocyte count in blood in 37 horses (Pr phenotypes without U and S) comprising part of the present material. Leucocyte count = X, and Pr protein concentration = Y.

The correlation coefficient (r) was 0.52.

The sample regression equation of Y on X was: $\hat{Y} = 5.28 X + 56.4$.

Figure 3 shows a scatter diagram of Pr protein values related to albumin concentration in the serum of 42 horses (Pr phenotypes without U and S alleles) in the present material.

The calculations were performed according to the methods described by Snedecor & Cochran (1967).



Figure 3. A scatter diagram of Pr protein values in relation to albumin concentration in serum in 42 horses (Pr phenotypes without U and S) comprising part of the present material. The albumin is expressed as X, and Pr protein concentration as Y.

The correlation coefficient (r) was -0.46.

The sample regression equation of Y on X was: $\hat{Y} = 169.5 - 21.5 \text{ X}.$

DISCUSSION

It has been reported that α_1 -antitrypsin concentration in human serum increases during bacterial infections and that after the intravenous administration of typhoid vaccine an increase to twice the normal levels has been registered (*Kueppers* 1968). The results in the present work indicate that the equine Pr protein reacts in a similar manner to human α_1 -antitrypsin, since Pr protein values of approx. double the normal ones were found in horses suffering from acute infections.

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In an earlier investigation, Pr protein levels were found to vary rather widely in healthy horses having the same Pr phenotype (Ek & Br @ nd 1980). A possible explanation for this could be that infections, though too slight to produce visible clinical symptoms, may influence the protease inhibitors. It seems also that Pr protein increases rapidly during inflammatory processes, thereafter quickly decreasing with recovery. Rapid fluctuations in the level of this protein may thus occur.

That there was a correlation between the Pr protein concentration and the leucocyte count is not surprising since leucocytes take part in inflammatory processes. It has been demonstrated that α_1 -antitrypsin inhibits the proteases contained in human granulocytes, in particular elastase and collagenase, the two enzymes considered to be the main ones responsible for the tissue destruction observed during inflammatory processes (Ohlson 1971).

In addition, a correlation was found between the Pr protein value and the serum albumin content, the Pr protein value increasing with decreasing albumin content. It is known that during acute inflammatory processes, an increased diffusion of albumin from the blood vessels takes place, resulting in a decrease in the serum concentration of albumin (*Thorén-Tolling* 1977).

Another disease group showing increased Pr protein values were horses with malignant tumours. In these cases an increased and uncontrolled growth of tissue cells is taking place leading to a high rate of tissue catabolism. The break down of protein in the organism is carried out by proteolytic enzymes. A high level of these proteases could explain an increase in the Pr protein value since this protein is a protease inhibitor. This is in accordance with findings in humans where elevated α_1 -antitrypsin levels were found in patients with non-resectable lung cancer. Patients with primary cancer at other sites also had elevated levels (*Harris et al.* 1974).

Acute laminitis is a condition connected with an aseptic inflammation in the corium of the hooves. Elevated Pr protein levels in these horses may thus be expected. The fact that it is a non-infective condition could explain the normal leucocyte count in these patients.

Investigations of serum antitrypsin activity in horses with chronic obstructive pulmonary disease have not revealed significant differences as compared with healthy horses (*Breeze et al.* 1977). Quantitative studies of different equine Pr phenotypes showed no types which could be compared with human Z-homo-zygotes in regard to the amount of Pr protein (Ek & Brænd). Finally, the results in the present work did not indicate that chronic obstructive pulmonary disease in horses is connected with a deficit of the Pr protein.

The human α_1 -antitrypsin increases rapidly in inflammatory conditions. The present work indicates that the equine Pr protein reacts in a similar manner. Testing of the Pr protein could therefore be a valuable supplement in laboratory clinical medicine especially in judging the seriousness and stage of development of infectious and inflammatory processes.

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SAMMENDRAG

Konsentrasjon av prealbumin (Pr) protein i serum hos syke hester og dets korrelasjon til antall leucocyter i blod og albumininnhold i serum.

Det ble foretatt en undersøkelse av Pr protein-konsentrasjonene i sera fra syke hester ved hjelp av *Mancini et al.*'s (1965) immundiffusjons-teknikk. Relative verdier i forhold til en valgt standard på 100 % ble bestemt på et totalantall på 102 hester. Hester med akutte infeksjoner hadde Pr protein verdier som var signifikant høyere enn normale dyr. Den høyeste individuelle verdi som ble registrert i denne gruppen var 202. Hester med akutt forfangenhet og maligne svulster hadde også forhøyede Pr protein verdier i forhold til normale dyr.

Det var en positiv korrelasjon mellom Pr protein verdien i serum og antallet leucocyter i blodet og en negativ korrelasjon mellom Pr protein verdien og albumininnholdet i serum.

(Received June 19, 1980).

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