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IMMUNE STATUS IN HORSES NATURALLY INFESTED WITH INTESTINAL PARASITES AND MANGE MITE

(With 5 Tables and 7 Figures)

By

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الحاله المناعيه فى الخيول المصابه طبيعياً بالطفيليات المعويه وظفيل الجرب

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اجرى الفحص الميكروسكوبى للعدد الكلى والنوعى لخلايا الدم البيضاء والفحص الباراسيتولوجى لحالات الطفيليات المعويه وظفيل الجرب. التحليل البيوكيميائى لسيرم الدم بالنسبه للبروتين الكلى والجزئى ومستوى فيتامين (هـ) تم اجراءه أيضاً. لم توجد فروق جوهريه فى العدد الكلى لكرات الدم البيضاء بين الخيول المريضه والكونترول بالرغم من ارتفاع اعداد خلايا الازيتوفيل فى حالات الاصابه المتوسطه والشديده لطفيليات الامعاء. انخفض العدد الكلى وخلايا النيتروفيل وازدادت اعداد خلايا الازيتوفيل فى حالة الاصابه بطفيل الجرب. لوحظ وجود نقص فى قيمة البروتين الكلى ونسبة الالبومين فى السيرم فى الخيول شديده الاصابه بالطفيليات الموجوده بالامعاء وظفيل الجرب بينما لم تظهر فروق جوهريه فى حالات الاصابه الطفيفه والمتوسطه لطفيليات الامعاء. لم توجد فروق جوهريه بين الخيول المريضه والخيول السليمه بالنسبه للجلوبولينات الألفا-1 أو الألفا-2. إلا أنه ظهرت فروق محدوده بين هذه المجاميع بالنسبه لجلوبولينات بيتا-1، بيتا-2 مع ظهور فروق جوهريه بالنسبه للجاما جلوبيولين. أظهرت الدراره وجود فروق جوهريه فى مستوى فيتامين هـ بين الخيول المريضه والسليمه. كما كانت هناك علاقه ايجابية بين النقص فى مستوى هذا الفيتامين وبين حده الاصابه بالطفيليات المعويه. لوحظ أيضاً وجود علاقه بين الانخفاض فى مستوى فيتامين هـ فى الخيول المريضه وازدياد نسبة الجاما جلوبيولين - ولقد اثبتت هذه الدراره وجود تأثيرات مجهده على كفاءة الجهاز المناعى للجسم وخاصة اذا كانت الاصابه بهذه العوامل شديده واستمرت لوقت طويل.

SUMMARY

Microscopic examination was done for total, and differential leukocytic count and parasitological examination for intestinal parasites and mange mite. Biochemical serum analysis for estimation of total protein, albumin, globulin, A/G ratio, electrophoresis and vitamin E was also performed. Total leukocytic count showed no significant difference due to intestinal parasitic infestation, although eosinophilia was found in moderate and heavy intestinal parasitic infestation. Leukopenia, neutropenia and eosinophilia were found in mange mite infection. Hypoproteinemia and hypoalbuminemia with significant decrease in A/G ratio were predominant in heavy intestinal infestation and in mange. Mild and moderate intestinal infestation showed no significant difference in serum total protein, although there was a low A/G ratio in moderate infestation. There was non-significant difference in alpha-1 and alpha-2 globulins between diseased and apparently healthy horses. However, there was non-significant increase in beta-1 and beta-2 globulins and significant increase in gamma globulin in horses with heavy intestinal infestation and with mange mite infection. A significant difference was found in serum levels of vitamin E between diseased and healthy control groups. There was a positive correlation between the drop of serum levels of vitamin E and the degree of intestinal parasitic infestation. A correlation was also found between serum levels of vitamin E and serum levels of gamma globulins. It can be concluded that the infection with intestinal parasites and mange mite, specially when it is heavy and prolonged for long time, has a marked stress effect on body immune efficiency.

Key Words: Horses-Intestinal Parasites Immune Status.

INTRODUCTION

The evaluation of immune status through estimation of serum total protein, albumin, A/G ratio and serum electrophoresis were suggested by James (1979). In addition Coles (1980) reported that alterations in plasma protein concentration may be indicative of diseases. The importance of vitamin E in enhancing of humoral immune response to both living and non-living antigens were reported by Lon (1982); Karen and Oalsrud (1986) and Bondich (1987). Tony (1991) stated that vitamin E is very important in animal diets and deficiencies occurring widely. The present study try to throw

a light on the immune status in relation to infection of horses with intestinal parasites and mange mite

MATERIALS and METHODS

Animals:

A total number of 76 horses ranging from 4 - 9 years old were examined in the present investigation. Most examined horses were owned by farmers in different villages belong to Assiut Governorate and some were examined during summer training courses for students in clinical semesters in 1996. Some horses showed emaciation, loss of weight, colic and diarrhea, while others showed diffuse areas of thick hairless skin lesions on the face and neck.

Among these examined horses, some appeared clinically healthy and showed no skin lesions. Case history including type and amount of feed was taken, where horses were fed on barley (concentrate), barseem (green forage) and wheat straw (dry roughage). All horses in the investigation were subjected to clinical examination according to Kelly (1974). Microscopic examination of faecal and skin scraps samples for detection of intestinal parasites and mange mites was done.

Faecal Samples:

Faecal samples were taken directly from the rectum and kept in clean plastic packs and transported directly to the laboratory for immediately microscopic examination using the concentration flotation technique. Counting of positive cases using McMaster technique to determine the degree of intestinal parasitic infestation was also done after Coles (1980).

Skin scraping:

Skin scraps and hairs were collected from the edges of the skin lesions at different localities and kept in clean plastic packs, then transported into centrifuge tubes, and KOH 10 % was added, then gentle heating and centrifugation at 3000 r.p.m. for 10 minutes. Microscopic examination of the sediment for mites was carefully done. Horses were divided after microscopic examination of faecal and skin scraps samples and before hematological examination and biochemical serum analysis into five groups as following:

A: Clinically apparently healthy control (n = 16)

B: Horses with mild intestinal parasitic infestation (n = 28)

C: Horses with moderate intestinal parasitic infestation (n = 12)

D: Horses with heavy intestinal parasitic infestation (n = 6)

E: Horses with mange mite infection (n = 10)

Blood Samples:

EDTA blood samples were used for total leukocytic count and for preparing of blood films stained with Giemsa stain (8 %) for differential leukocytic count using the four field meander system (Coles, 1980). Blood samples without anticoagulant were used for obtaining clear, non-haemolysed serum, which transported into clean, dry and dark vials for immediately estimation of serum level of vitamin E (Hawk et. al., 1954) and serum electrophoresis (Ritzman and Daniels, 1979). The rest of serum samples were kept frozen at -20 °C for determination of total protein and albumin spectrophotometry using chemical kits supplied by Bicon (Germany) after the method of Henry and Webster (1964).

RESULTS

Microscopic examination of all collected faecal samples for intestinal parasites was positive for 46 horses, while 30 horse were negative, from these 16 horses appeared clinically healthy and used as control and the rest (14 horses) showed diffuse, irregular, thick hairless areas at the face and neck (Fig. 6), from these ten horses were positive for mange mite (*Psoroptic* sp.-Fig. 7). The other 4 horses with similar skin lesions and with unknown cause were excluded from other investigations. The positive intestinal parasitic infestation were divided according to egg counting into mild (n = 28), where egg count was less than 600 egg/gm faeces to moderate (n = 12) with egg count between 600 - 1000 egg/gm faeces and heavy infestation (n = 6), where egg count was more than 1000 egg/gm faeces.

The results of total and differential leukocytic count in control and diseased groups are summarized in table 1, where total leukocytic count showed no significant difference due to intestinal parasitic infestation, although eosinophilia was found in moderate and heavy infestation. leukopenia and eosinophilia were found in mange mite infection.

Biochemical serum analysis of proteins are summarized in table 2 and figure 1, where significant hypoproteinaemia, hypoalbuminaemia, decrease in A/G ratio and significant hyperglobinaemia were predominant in heavy parasitic infestation and in mange mite infection. Mild and moderate

intestinal parasitic infestation showed no significant difference in serum total protein, although there was a low A/G ratio in moderate infestation.

Serum electrophoresis results are summarized in table 3 and figure 2, where there was no significant difference in alpha-1 and alpha-2 between diseased and control groups. There was non-significant increase in beta-1 and beta-2 and significant increase in gamma globulin in diseased groups (D&E) if compared with apparently healthy group (A).

Serum levels of vitamin E in diseased and control groups are summarized in table 4 and figure 3, where there was a significant change between these groups. The decline in the level of vitamin E was correlated with the severity of intestinal parasitic infestation. In addition, this decline was lower in mange mite infection than in intestinal parasitic infestation. It was also found that there was a correlation between the increase in gamma-globulin and the decrease in serum level of vitamin E, where there was a higher drop in serum vitamin E with higher increase in gamma-globulin in heavy intestinal parasitic infestation and lower drop in vitamin E with lower increase in gamma-globulin in mange mite infection (table 5 & figure 4).

DISCUSSION

The changes in total leukocyte count were non-significant in intestinal parasitic infestation, although eosinophilia was observed in moderate and heavy infestation. Patrick et. al., (1991) found that the average number of leukocyte in adult horses was $6 - 12 \times 10^3$ /cmm. The leukopenia, neutropenia and eosinophilia in mange mite infection reflect the suppressive effect of external parasitic infection with mange mite.

The changes in serum total protein and albumin in mild and moderate intestinal parasitic infestation (table 2) were not significant if compared with those of apparently healthy control ones. However, Patrick et. al., (1991) reported that the serum total protein increase in horses suffer from intestinal parasitic infestation due to the increase in production of all globulin fractions. Knottenbelt (1992) found that the main values of serum total protein and albumin in horses with intestinal parasitic infestation were 6.25 - 7.0 gm/dl and 2.7 - 3.65 gm/dl respectively. The recorded hypoproteinaemia and hypoalbuminaemia in horses, groups D and E, may caused by impaired intestinal absorption of nutritive substances due to injury of the intestinal mucosa by parasitic infestation. This can be supported by Sally et. al., (1986), who found marked drop in serum total protein (4.5 gm/dl) in adult horses suffered from diarrhea caused by *Strongylus* sp. infestation. In

addition Swell and Brocklesby (1990) and Eugen et. al., (1996) reported a decrease in serum albumin in horses infested with *Strongylus* parasites. Furthermore, in mange mite infection (group E) this decline in serum levels of total protein and albumin can be attributed to rapid breakdown of serum total protein resulting from continuous movement during rapping of the skin lesions due to severe itching. In relation to the decrease in serum albumin in this group, this may be caused by the increase in serum globulin in response to invasion of the skin with mange mite. Moragg (1991) stated that large animals particularly horses associated with heavy parasitic burden, have decreased serum total protein and albumin concentration.

There was an increase in serum globulin associated with significant decrease in A/G ratio in moderate and heavy intestinal infestation (C&D) and mange mite infection (E) if compared with apparently healthy control (A) (table 2 & figure 1). This can be supported by Knottenbelt (1992), who found that the normal value of serum globulin in horses was 1.7- 4.0 gm/dl and A/G ratio was 0.9 - 1.5. The increase in serum globulin in the present investigation can be attributed to the response of immune system of the diseased horses against the invasion of the body with intestinal parasites and mange mite, or may be compensatory to the hypoalbuminemic state in these diseased animals. Swell and Brocklesby (1990) found an increase in serum globulin in equids, naturally infested with *Strongylus* parasites. In addition, Josiel et. al., (1992) reported that serum globulin was increased in weight loss horses infested with gastrointestinal parasites.

Serum protein electrophoresis showed no significant changes in alpha-1 and alpha-2 globulins, between diseased and apparently healthy groups (table 3 & figure 2). This can reflect the chronicity of the condition in diseased horses. Joan (1982) and Robert and Keith (1986) stated that alpha-1 and alpha-2 globulins tend to increase when there is active tissue damage (acute phase reaction). There was non-significant increase in beta globulins with significant increase in gamma globulin in heavy intestinal parasitic infestation (D) and in mange mite infection (E) if compared with apparently healthy control (A). This can explain the immune response of the animal body against its invasion with intestinal parasite or mange mite. Beta globulin is often increased in association with increase in gamma globulin, when an intense immune response is in effect (Robert and Keith, 1986). In addition, Coles (1980) reported that alteration in gamma globulin usually reflects a response of reticuloendothelial system to antigenic stimulation, where infections accompanied by invasion of the body by foreign material- whatever it be of

bacterial, viral, protozoal or parasitic origin- usually result in increase in concentration of gamma globulin.

There was significant decrease in serum levels of vitamin E in diseased horses than in apparently healthy ones. There was a higher drop in horses with intestinal parasites than those with mange mite infection. This changes may be due to the high continuous consumption of the animal body for this vitamin to assist its resistance against the invasion with these antigens (intestinal parasites and mange mite). The higher drop of vitamin E level on intestinal parasitic infestation may be due to the impaired absorption through the intestinal mucosa due to severe injuries resulting from heavy intestinal infestation, moreover the competitive demand of intestinal parasites for this vitamin can aggravate the loss in serum level of the vitamin. This can also explain the correlation between the decrease in serum level of vitamin E and the severity of intestinal infestation. Horses need a high level of vitamin E to increase their immunity against infectious diseases (Lon, 1982). The decrease in serum vitamin E with increase of serum gamma globulin in heavy intestinal parasitic infestation reflect a severe degree of diseased condition and explain the high consumption of vitamin E, when there is a high degree of antigenic stimulation represented by high titer of gamma globulin.

It can be concluded that the infestation of horses with intestinal parasites and mange mite, specially when it is heavy and prolonged for long time has a marked stress effect on the body immune efficiency. Improving the level of vitamin E in association with adequate protein supplementation for these diseased horses can minimize the risk of complications resulting from the suppressive effect of these agents on the immune status of the body and keep the horses in good performance.

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Table 1 : Values # of total and differential leucocytic count in horses naturally infected with intestinal parasites and mange mite and healthy control ones.

Groups	Number of animals	WBC's X 10 ³ /Cu ²	Neutrophil %	Lymphocyte %	Eosinophil %	Basophil %	Monocyte %
A	16	7.94 ± 0.17	58.97 ± 0.68	35.77 ± 0.60	2.60 ± 0.16	1.05 ± 0.17	1.61 ± 0.16
B	28	7.88 ± 0.31	58.58 ± 0.52	36.07 ± 0.16	2.85 ± 0.12	0.86 ± 0.03	1.64 ± 0.08
C	12	8.02 ± 0.21	59.47 ± 0.94	30.60 = 1.46	7.55 ** ± 0.44	0.80 ± 0.20	1.58 ± 0.12
D	6	7.83 ± 0.16	58.78 ± 0.91	30.18 ± 1.02	8.28 ** ± 0.13	0.72 ± 0.16	2.04 ± 0.10
E	10	5.15 ** ± 0.87	44.40 ** ± 0.73	30.80 ± 1.15	18.77 ** ± 2.70	1.77 ± 0.17	4.26 ± 0.25

: Mean = standard error

* : P < 0.05

** : P < 0.01

A : Healthy control horses.

B : Horses with mild intestinal parasitic infestation.

C : Horses with moderate intestinal parasitic infestation.

D : Horses with heavy intestinal parasitic infestation.

E : Horses with mange mite infection.

Table 2 : Values[#] of serum total protein, albumin and A/G ratio in horses naturally infected with intestinal parasites and mange mite and healthy control ones.

Groups	Number of animals	Total Protein gm/dl	Albumin gm/dl	Globulin gm/dl	A/G ratio
A	16	6.42 ± 0.24	2.70 ± 0.14	3.72 ± 0.24	0.73 ± 0.05
B	28	6.49 ± 0.29	2.60 ± 0.19	3.89 ± 0.39	0.67 ± 0.15
C	12	6.54 ± 0.11	2.52 ± 0.13	4.02 * ± 0.20	0.63 ± 0.12
D	6	5.72 ** ± 0.18	1.69 ** ± 0.14	4.03 ** ± 0.22	0.42* ± 0.09
E	10	5.82** ± 0.13	1.88** ± 0.08	3.94 * ± 0.25	0.48* ± 0.07

: Mean = standard error * : P < 0.05 ** : P < 0.01
 A : Healthy control horses. B : Horses with mild intestinal parasitic infestation.
 C : Horses with moderate intestinal parasitic infestation.
 D : Horses with heavy intestinal parasitic infestation.
 E : Horses with mange mite infection.

Table 3: Values # of protein electrophoresis in horses naturally infected with intestinal parasites and mange mite and healthy control ones

Groups	Number Of Animals	Albumin		Globulin		Alpha 1		Alpha 2		Beta 1		2 Beta		Gamma	
		%	Gm/dl	%	Gm/d	%	Gm/dl	%	Gm/d	%	Gm/dl	%	Gm/dl	%	Gm/dl
A	16	42.06	2.70	57.94	3.72	17.13	1.10	6.07	0.39	7.01	0.45	3.89	0.25	23.83	1.53
		±0.24	±0.14	±1.93	±0.24	±0.20	±0.15	±1.45	±0.08	±2.62	±0.16	±2.25	±0.11	±2.0	±0.17
D	6	29.5	1.69	70.5	4.03	16.60	0.95	6.29	0.36	8.39	0.48	6.29	0.36	32.8	1.88
		±0.37	±0.14	±2.37	±0.22	±0.23	±0.18	±1.24	±0.07	±2.76	±0.06	±0.99	±0.08	±2.25	±0.13
E	10	32.3	1.88	67.7	3.94	15.5	0.98	5.69	0.42	8.54	0.48	5.69	0.32	29.18	1.74
		±0.32	±0.08	±1.83	±0.26	±0.23	±0.14	±1.14	±0.10	±2.64	±0.14	±1.27	±0.06	±2.23	±0.15

: Mean ± standard error * : P < 0.05 ** : P < 0.01

A : Healthy control horses.

D : Horses with heavy intestinal parasitic infestation.

E : Horses with mange mite infection.

Table 4: Values # of serum levels of vitamin E in horses naturally infected with intestinal parasites and mange mite and healthy control ones.

Groups	Number of animals	Vitamin E ($\mu\text{g/ml}$)
A	16	1.41 \pm 0.39
B	28	0.89 \pm 0.27 *
C	12	0.64 \pm 0.22 **
D	6	0.51 \pm 0.24 **
E	10	0.92 \pm 0.30 *

: Mean \pm standard error

* : P < 0.05

** : P < 0.01

A : Healthy control horses.

B : Horses with mild intestinal parasitic infestation.

C : Horses with moderate intestinal parasitic infestation.

D : Horses with heavy intestinal parasitic infestation.

E : Horses with mange mite infection.

Table 5: Correlation between serum levels of vitamin E and gamma globulin in horses naturally heavy infested with intestinal parasites and mange mite infection and healthy control ones.

Groups	Number of animals	Vitamin E ($\mu\text{g/ml}$)	Gamma- globulin (gm/dl)
A	16	1.41 \pm 0.39	1.53 \pm 0.17
D	6	0.51 \pm 0.24	1.88 \pm 0.13
E	10	0.92 \pm 0.30	1.74 \pm 0.15

A : Healthy control horses..

D : Horses with heavy intestinal parasitic infestation.

E : Horses with mange mite infection

Fig.1: Serum total protein, albumin, globulin and A/G ratio in diseased and control horses.

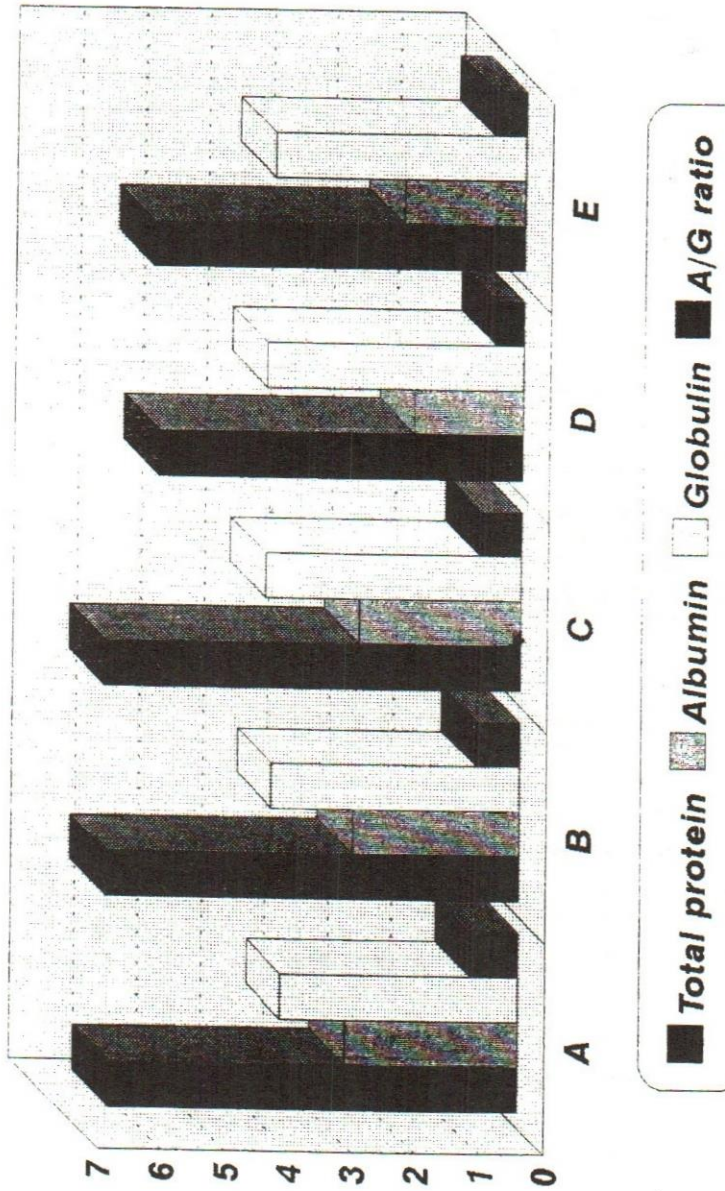


Figure 2 : Serum electrophoresis in healthy and diseased horses

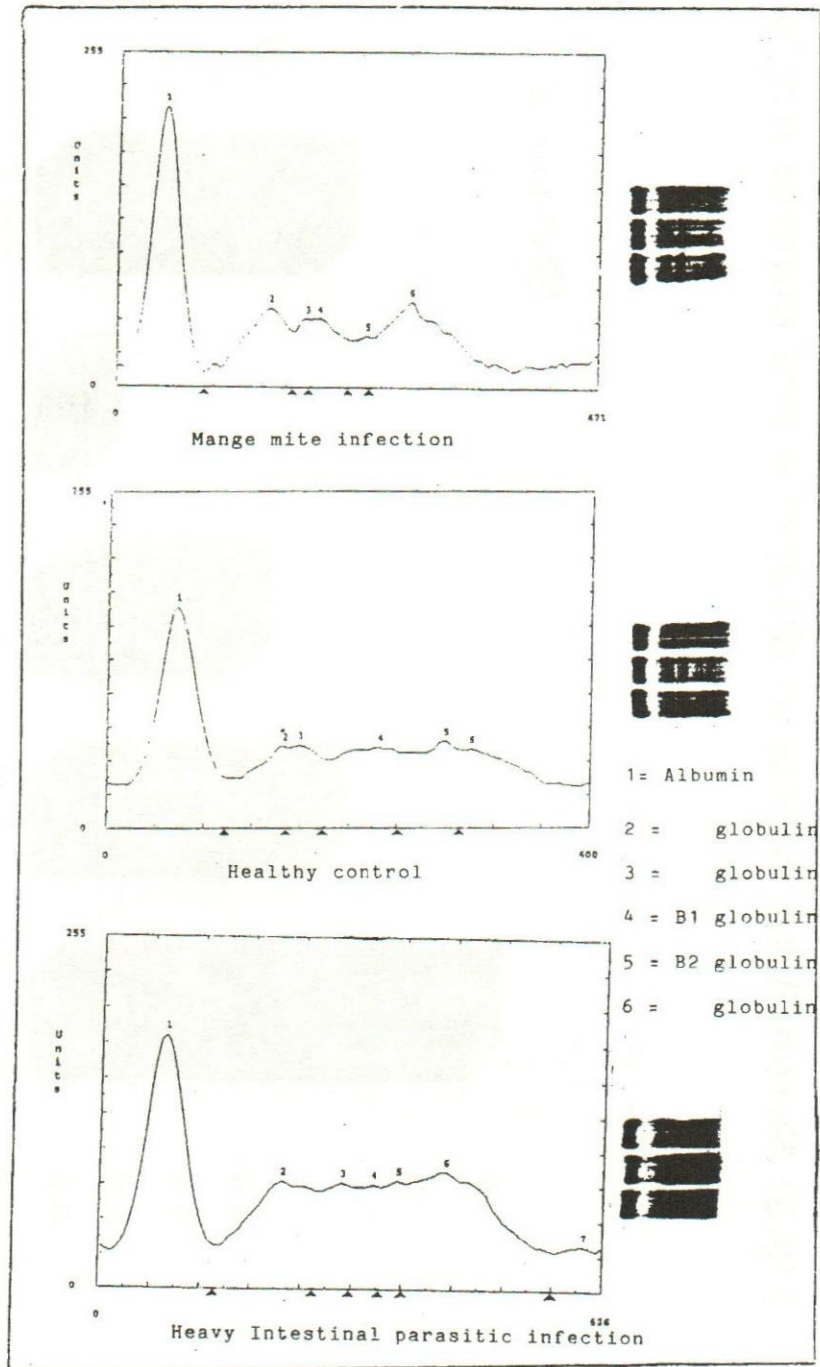


Fig.3: Serum Vit.E levels in diseased and control horses.

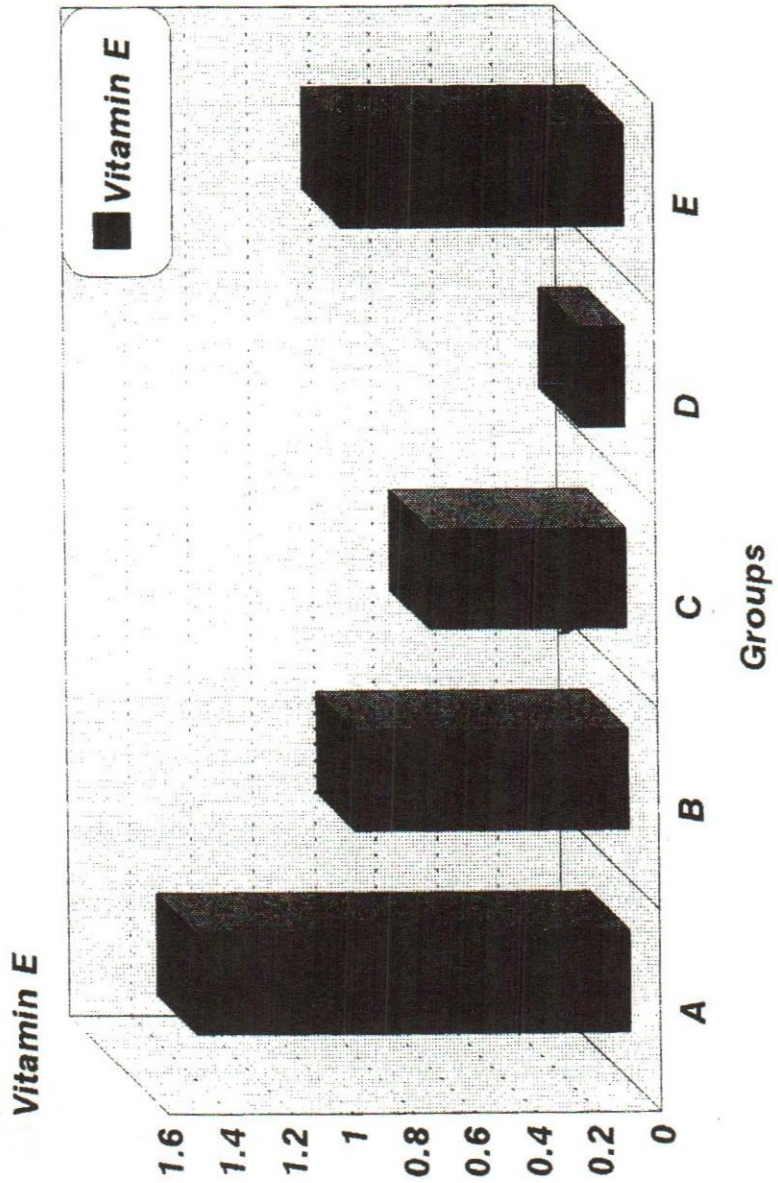


Fig.4: Correlation between serum Vit.E and gama globulin in healthy and diseased horses.





Figure 5: A horse with heavy intestinal parasitic infestation. Note the severe emaciation.



Figure 6: A horse with skin lesions (mange) at the face and neck.

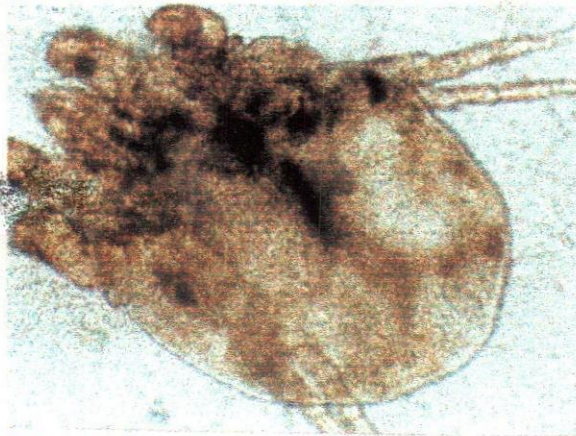


Figure 7: Female *Psoroptic* sp. showing suckers on long jointed stalks (200 X).