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SERUM PROTEIN FRACTIONS AS DETERMINED BY CELLULOSE ACETATE ELECTROPHORESIS IN *SCHISTOSOMA MATTHEEI* INFESTED SHEEP

LULU C. VAN ZYL, Veterinary Research Institute, Onderstepoort

ABSTRACT

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The serum protein fraction values of Dorper sheep infested with *Schistosoma mattheei* were determined by cellulose acetate electrophoresis at regular intervals for 15 months. Statistical analyses showed that increases in γ globulin and decreases in albumin were significant.

INTRODUCTION

Bilharziasis is an important and widespread disease of ruminants in the Republic of South Africa (Reinecke, 1970) and according to McCully & Kruger (1969) it is mainly caused by *Schistosoma mattheei*. The parasitological and pathological features of *S. mattheei* infestation in sheep have been investigated by Le Roux (1929) and McCully & Kruger (1969), but the only clinico-pathological information available is that obtained by Malherbe (1970) in a limited paper electrophoretic study on serum.

This investigation was conducted to obtain more detailed quantitative information on the dynamics of plasma protein fractions of sheep infested with *S. mattheei*. Cellulose acetate electrophoresis was the method chosen because of its accuracy, rapidity and repeatability, as shown by a previous investigation on the serum of normal sheep (Van Zyl, 1967). According to Stauber (1954) cellulose acetate electrophoresis has been applied only rarely to the study of trematode infestation, viz. *Schistosoma mansoni* in mice.

MATERIALS AND METHODS

Experimental animals

Twenty-two Dorper sheep were reared helminth-free and maintained under controlled conditions for the duration of the experiment.

Twelve of the sheep were wethers 12½ to 13½ months old, and 10 were male lambs 4½ to 6 months old at the beginning of the experiment. Eight wethers and seven male lambs were infested while four wethers and three lambs acted as uninfested controls.

The animals received a daily ration of 227 to 455 g of a concentrate mixture consisting of 32,27 kg yellow maize meal, 9,09 kg lucerne meal, 2,27 kg blood meal, 910 g bone meal, 455 g salt and 455 g urea. In addition each sheep received approximately 455 g teff hay each day.

Infestation

An attempt was made to simulate the natural course of bilharziasis. Hence sheep were not infested with massive doses of cercariae as this would probably have resulted in an acute condition.

With the exception of one wether, which received cercariae through an incision in the groin, the sheep were infested percutaneously via the fore limbs, back or groin with 2 000 to 6 000 cercariae each (J. A. van Wyk, personal communication, 1973).

Examination of serum proteins

Serum protein determinations were conducted at four to six weekly intervals commencing one month after infestation and continued for 15 months.

Collection of serum: Animals were bled between 08h00 and 12h00 by jugular venipuncture. The blood was left at room temperature for 20 min and then centrifuged at 3 000 rpm for 15 min. The serum thus obtained was tested immediately.

Analytical methods: Total serum protein (TSP) determinations were carried out by the biuret method of Weichselbaum (1946). The serum protein fractions were separated electrophoretically on cellulose acetate membranes with a Beckman Microzone apparatus. The membranes were stained, subsequently scanned on the Beckman model RB Analytrol Densitometer and the serum protein fractions determined quantitatively according to the manufacturer's instructions (Anon., 1957, 1965).

Statistical analyses

Statistical analyses were carried out according to the Wilcoxin's two sample test (Steel & Torrie, 1960), with a 5 per cent level for significant and a 1 per cent level for highly significant differences. The infested and uninfested sheep were compared for total serum protein, individual serum protein fractions and albumin/globulin ratios.

RESULTS

Infestation in these sheep was proved by the presence of viable schistosome ova in their faeces according to the method described by Kruger & Heitmann (1967). The maximum number of miracidia which hatched from the faeces of these animals varied from 63 to 3 323 per 5 g of faeces. The prepatent period was approximately 45 days (J. A. van Wyk, personal communication, 1973).

Nine infested sheep survived for the duration of the experiment, but six died: at 4½ months one, at six months two and at 13½ months one, wether died, while one lamb died at 10 months and another at 14 months.

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Serum proteins

Total serum proteins: TSP values varied little for some months; a significant increase occurred after 10 months and persisted until the end of the experiment (Table 1).

TABLE 1 Mean TSP values

Months after infestation	TSP (g%)		T value
	Infested sheep	Non-infested sheep	
1	6,86	7,00	96
2	6,66	6,98	103
3	6,25	6,76	107,5
4	6,74	6,88	91
5	6,08	6,23	74,5
5½	6,65	7,06	82
6½	7,05	6,98	68,5
7½	7,71	7,11	51,5
8½	7,79	7,25	47
9	7,03	6,63	50,5
10	8,19	7,18	40,5*
11	7,72	6,65	40*
12	8,00	6,77	35**
14	8,56	7,40	40,5*
16	8,44	7,30	36*

* Significant difference
** Highly significant difference

Further analysis of the results revealed that the significant increases in the TSP values were actually caused by increases in this value in the lambs; the increases in the wethers were not significant. Moreover, the values for the infested lambs were significantly increased a month before those of the infested sheep as a whole group (Table 2).

TABLE 2 Mean TSP values for infested and non-infested wethers and lambs

Months after infestation	Wethers			Lambs		
	TSP (g%)		T value	TSP (g%)		T value
	Infested	Non-infested		Infested	Non-infested	
8½	7,62	7,47	18,5	7,91	6,96	7,5
9	6,75	6,81	23,5	7,24	6,38	6*
10	7,81	7,41	14	8,46	6,88	6*
11	7,39	6,90	16	8,00	6,32	7*
12	7,54	6,93	14	8,39	6,56	6*
14	7,94	7,53	17,5	9,08	7,22	6*
16	7,85	7,36	15	8,91	7,21	6*

* Significant difference

Albumin: One of the most marked changes in the serum protein content was the decrease in the values for serum albumin. Two months after infestation, i.e. shortly after the onset of patency, a significant difference was apparent (Table 3). With the exception of the values obtained $7\frac{1}{2}$ months after infestation, the differences between infested and control sheep were significant until nine months after infestation, when they became highly significant, a situation that persisted until the end of the experiment.

TABLE 3 Mean serum albumin values of infested and non-infested sheep

Months after infestation	Serum albumin (g%)		T[1] value
	Infested sheep	Non-infested sheep	
1	4,29	4,29	80
2	3,82	4,35	46,5*
3	3,24	4,16	34**
4	3,49	4,37	31**
5	2,76	3,67	28,5**
$5\frac{1}{2}$	3,05	3,80	47**
$6\frac{1}{2}$	3,12	3,83	31**
$7\frac{1}{2}$	3,33	3,60	52
$8\frac{1}{2}$	3,64	4,16	46*
9	3,16	3,85	33**
10	3,42	4,36	32**
11	3,03	3,96	30**
12	3,01	3,91	30**
14	3,02	4,44	28**
16	2,66	3,76	29**

* Significant difference
** Highly significant difference

α_1-*globulin fraction*: Significant increases in the α_1-globulin fraction occurred five months after infestation (Table 4). The values remained elevated, albeit not statistically significant, until three months later when a highly significant increase was recorded. Hereafter the values levelled off, the differences being slight in most cases until the end of the experiment.

TABLE 4 Mean α_1-globulin values

Months after infestation	α_1-globulin (g%)		T value
	Infested sheep	Non-infested sheep	
1	0,33	0,35	91,5
2	0,33	0,36	90
3	0,35	0,33	69,5
4	0,34	0,28	65
5	0,39	0,31	41,5*
$5\frac{1}{2}$	0,38	0,33	55,5
$6\frac{1}{2}$	0,40	0,36	53,5
$7\frac{1}{2}$	0,38	0,38	74
$8\frac{1}{2}$	0,40	0,23	35**
9	0,33	0,27	41,5*
10	0,34	0,31	65,5
11	0,37	0,29	48
12	0,35	0,29	46
14	0,35	0,28	41*
16	0,44	0,44	61

* Significant difference
** Highly significant difference

α_2-*globulin fraction*: The α_2-globulin values were significantly elevated three months after infestation (Table 5). With the exception of highly significant values at 10 and 12 months, only slight increases were recorded at other times.

TABLE 5 Mean α_2-globulin values

Months after infestation	α_2-globulin (g%)		T value
	Infested sheep	Non-infested sheep	
1	0,78	0,78	88
2	0,82	0,70	52
3	0,75	0,64	42,5*
4	0,77	0,71	70,5
5	0,70	0,64	50,5
$5\frac{1}{2}$	0,76	0,74	64
$6\frac{1}{2}$	0,80	0,74	54
$7\frac{1}{2}$	0,87	0,83	58
$8\frac{1}{2}$	0,82	0,82	64
9	0,73	0,72	71,5
10	0,86	0,74	32**
11	0,78	0,71	47,5
12	0,79	0,67	36**
14	0,85	0,81	61
16	0,80	0,80	82

* Significant difference
** Highly significant difference

β-*globulin fraction*: Two months after infestation the β globulin values showed a significant increase. No further statistically significant results were recorded until 10 months after infestation, when highly significant increases were recorded on four occasions.

TABLE 6 Mean β-globulin values

Months after infestation	β-globulin (g%)		T value
	Infested sheep	Non-infested sheep	
1	0,47	0,58	113,5
2	0,49	0,42	42*
3	0,50	0,46	65
4	0,47	0,40	58,5
5	0,54	0,48	48,5
$5\frac{1}{2}$	0,59	0,55	61
$6\frac{1}{2}$	0,77	0,67	55,5
$7\frac{1}{2}$	0,69	0,59	49,5
$8\frac{1}{2}$	0,58	0,50	50
9	0,59	0,48	49
10	0,74	0,48	35**
11	0,71	0,44	33,5**
12	0,74	0,45	35,5**
14	0,82	0,56	31**
16	0,67	0,60	47,5

* Significant difference
** Highly significant difference

Main γ-globulin fraction: The main γ-globulin fraction of the infested sheep showed the most marked increases encountered in this study (Table 7). Highly significant increases were recorded four months after infestation and the values remained at this level for most of the experimental period.

TABLE 7 Mean main γ-globulin values

Months after infestation	Main γ-globulin (g%)		T value
	Infested sheep	Non-infested sheep	
1	0,85	0,88	89,5
2	1,10	1,00	62,5
3	1,28	0,99	52
4	1,61	1,00	35**
5	1,59	1,06	30,5**
5½	1,81	1,33	49,5*
6½	1,90	1,39	49,5*
7½	2,34	1,48	40**
8½	2,28	1,41	40**
9	2,15	1,17	32**
10	2,80	1,19	28**
11	2,75	1,07	28**
12	3,05	1,26	28**
14	3,41	1,12	28**
16	3,63	1,43	28**

* Significant difference
** Highly significant difference

A more detailed analysis of the data revealed that the infested lambs showed a significant increase in γ-globulin values two months after infestation (early patency), i.e. two months before the increases became statistically significant in the group as a whole (Table 8). The increases in the values for wethers, however, became statistically significant only nine months after infestation.

TABLE 8 Detailed analysis of mean main γ-globulin values for infested and non-infested wethers and lambs

Months after infestation	Wethers			Lambs		
	Main γ-globulin (g%)		T value	Main γ-globulin (g%)		T value
	Infested	Non-infested		Infested	Non-infested	
1	0,83	0,96	36	0,87	0,78	11
2	1,03	1,06	28	1,19	0,91	6*
3	1,19	1,01	25,5	1,39	0,94	6*
4	1,54	1,08	15	1,69	0,90	6*
5	1,53	1,10	11,5	1,67	1,03	6*
5½	1,52	1,34	19	2,09	1,32	6*
6½	1,49	1,46	21,5	2,25	1,30	6*
7½	1,82	1,43	16	2,71	1,54	6*
8½	1,94	1,42	16	2,52	1,40	6*
9	1,84	1,23	10*	2,36	1,08	6*
10	2,52	1,23	10*	3,00	1,13	6*
11	2,47	1,09	10*	2,98	1,04	6*
12	2,79	1,31	10*	3,27	1,20	6*
14	3,08	1,12	10*	3,70	1,10	6*
16	3,50	1,47	10*	3,74	1,38	6*

* Significant difference

Trailing γ-globulin: These values fluctuated considerably and the results showed no definite trend (Table 9).

TABLE 9 Mean trailing γ-globulin

Months after infestation	Trailing γ-globulin (g%)		T value
	Infested sheep	Non-infested sheep	
1	0,15	0,13	67,5
2	0,10	0,17	93,5
3	0,13	0,17	87
4	0,04	0,02	74
5	0,07	0,07	63
5½	0,07	0,25	82,5
6½	0,07	0,13	88
7½	0,10	0,23	92,5
8½	0,07	0,13	74
9	0,09	0,14	86
10	0,04	0,10	86
11	0,07	0,19	93,5
12	0,06	0,19	92,5
14	0,11	0,20	82
16	0,24	0,16	49,5

Albumin/globulin (A/G) ratio: These values showed a consistent decrease and differences between the two groups became highly significant three months after infestation (Table 10).

Overall comparison of results

The albumin, main γ-globulin and A/G ratio values showed significant differences early in the experiment while those of TSP and β-globulin did so some time later. The mean values for TSP, albumin and main γ-globulin are illustrated in Fig. 1.

This clearly shows that fluctuations in the albumin and globulin fractions complemented each other until 10 months after infestation. The TSP values of infested animals showed significant increases from 11 months after infestation, when increases in the main γ-globulin values were greater than the decreases in albumin and resulted in a significant increase in TSP.

TABLE 10 Mean albumin/globulin ratio

Months after infestation	Infested sheep (g%)	Non-infested sheep (g%)	T value
1	1,67	1,60	102,5
2	1,39	1,67	52,5
3	1,10	1,61	30**
4	1,12	1,77	33**
5	0,87	1,43	26**
5½	0,90	1,26	43,5*
6½	0,84	1,13	44,5*
7½	0,80	1,03	41,5*
8½	0,93	1,36	35**
9	0,85	1,40	28**
10	0,78	1,50	28**
11	0,71	1,48	28**
12	0,64	1,37	28**
14	0,56	1,51	28**
16	0,50	1,07	28**

* Significant difference

** Highly significant difference

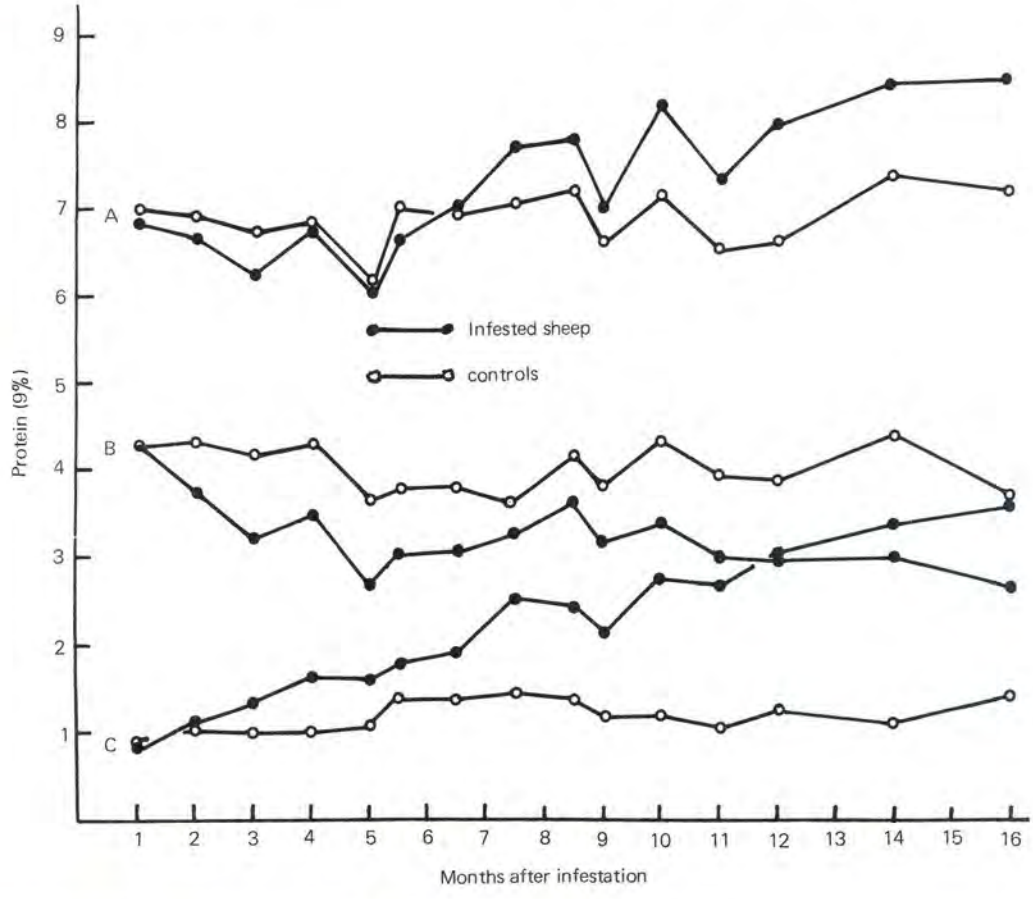

FIG. 1 Comparison between mean values for TSP, albumin and main γ-globulin fractions

A = TSP B = Albumin C = Main-globulin

Discussion

The most significant pathological changes in ovine bilharziasis have been ascribed to the presence of ova and dead schistosomes in the intralobular and intrahepatic branches of the portal vein (McCully & Kruger, 1969). Symmers (1904) also described liver damage in human beings suffering from bilharzia.

There is evidence that the host reaction to schistosome ova is a manifestation of hypersensitivity. Andrade & Cheever (1967) postulated that a delayed type of hypersensitivity occurs in some cases of *Schistosoma mansoni* and that it is represented by an active hepatitis with no correlation between the intensity of the reaction and the number of parasites. Warren, Domingo & Cowan (1967) found an immunological reaction of the delayed hypersensitive type in mice sensitized with *S. mansoni* eggs. They showed that this sensitivity could be transferred to other mice by cells but not by serum.

Lowered serum albumin and elevated γ-globulin values have been recorded in Laennec's cirrhosis by Leutscher (1940, 1941) and liver disease in general by Gray & Barron (1943). Similar changes were observed in bilharziasis due to infestation with *Schistosoma mansoni* in man (Aufses, Schaffner, Rosenthal & Herman, 1959; Mousa, Atta, El Rooby, El Garem, Saif & Zein-El Abdin, 1966; Coutinho, 1968); *Schistosoma bovis* in lambs (Lengy, 1962) and *S. mattheei* in sheep (Malherbe, 1970).

The electrophoretic serum protein patterns observed in the present study provide further evidence that liver damage is the overriding pathogenic factor in bilharziasis.

The low serum albumin concentrations obtained indicate that the capacity of the liver to synthesize albumin may be impaired. According to Starling (1896) albumin is particularly important in the maintenance of the osmotic pressure of the blood and is therefore probably an important factor in the development of the ascites, hydrothoriax and hydropericardium encountered in animals with bilharziasis (McCully & Kruger, 1969).

The rise in TSP values observed in this investigation occurred because the net gain in the total globulin fraction was greater than the loss of albumin. It seems improbable, however, that the increase in serum γ-globulin was compensatory for the depletion of albumin. The colloidal osmotic pressure of the γ-globulins is probably too low to be of significant aid in the balance of intra- and extravascular fluid. Moreover, an increase in γ-globulins may be sustained long after the return of the serum albumin level to normal (Thorn, Armstrong, Davenport, Woodruff & Tyler, 1945).

The significant increase in the TSP values of the infested animals was due to increases in the TSP values of the lambs. In the infested wethers the rises in TSP did not reach significant levels during the period of this investigation. Whether significant differences in the TSP concentrations in these animals would have been discernible if the experiment had been prolonged is open to conjecture. It seems that the adult sheep is more capable of stabilizing its total serum protein concentration than the young animal.

The main γ-globulin fraction, which showed significant increases in the lambs long before it occurred in the older animals, was mainly responsible for the significant increases in the TSP values. This may be due to the presence of a more active immune system in the younger animals.

Slight, but inconsistent, increases in α_2-globulin were also noted in this study. Leland (1961) considered increases in this serum fraction to be in keeping with the general concept that α-globulin increases in conditions of inflammation or tissue destruction, irrespective of the cause.

In nephrosis the serum protein pattern is one of lowered serum albumin and γ-globulin fractions and lowered TSP values (Malmros & Blix, 1946; Lewis & Page, 1947). This is in striking contrast to the serum protein picture found in this study which confirms the observations of McCully & Kruger (1969) that kidney lesions are rarely present in sheep suffering from bilharziasis.

Leutscher (1941) conducted electrophoretic studies on patients with lobar pneumonia with pleural effusion. The α and β-globulin values were markedly increased while the γ-globulin values showed a lesser rise; the TSP values declined slightly but there was a considerable decrease in albumin. This differs from the present observations in which pneumonia was found in only one animal.

In this investigation the serum protein fractions of infested sheep did not show changes which may be considered characteristic of bilharziasis.

The serum protein patterns obtained must, however, be considered as integral parts of the clinical picture as a whole; the changes are symptomatic and not pathognomonic. They implicate liver impairment as being the primary factor concerned and indicate that renal and pulmonary involvements are not significant features of the disease.

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