SERUM PROTEIN CONCENTRATIONS IN CATTLE EXPERIMENTALLY INFECTED WITH *Trypanosoma evansi*

PROTEINOGRAMA SÉRICO DE BOVINOS EXPERIMENTALMENTE INFECTADOS COM *Trypanosoma evansi*

M. C. A. TEIXEIRA[1], L. C. MARQUES[1*], J. J. FAGLIARI[1], R. Z. MACHADO[1], P. C. SILVA[1], E. S. PORTUGAL[1], C. E . P. SANTOS[1], A. M. GIRARDI[1]

SUMMARY

Trypanosoma evansi is pathogenic to several animal species in tropical and subtropical areas. In Brazil, the disease is endemic in Pantanal mato-grossense. This study aimed to determine the electrophoretic profile of serum proteins in experimentally infected cattle. Eight crossbred animals, aged approximately eight months, clinically healthy and seronegative (IFAT) for *Tripanosoma evansi* were used. The animals were divided into two groups, G1: five animals inoculated intravenously with 22 x 10^6 trypomastigotes of *T. evansi*, and G2: three animals kept as controls. The blood used to obtain serum samples was collected daily until the 15th day after inoculation (DAI), and subsequently, at weekly intervals until the 204th DAI, every 14 days until the 372nd DAI and every 30 days until the 522nd DAI. Electrophoresis in polyacrylamide gel containing sodium dodecyl sulfate (SDS-PAGE) was used for the purification of proteins. Through the biological test, *T. evansi* was detected in animals 01, 06 and 08 on the 15th DAI, 06 and 07 on the 30th DAI, 01 and 06 on the 45th DAI, 06 on the 60th DAI and 01 on the 75th DAI. Twenty-six proteins with molecular weights ranging from 20 kDa to 245 kDa were found in cattle. Eight of them were nominally identified as immunoglobulin A (IgA), ceruloplasmin, transferrin, albumin, heavy and light chain immunoglobulin G (IgG), haptoglobin and acid glycoprotein.

KEY-WORDS: Cattle. Protein electrophoresis. Trypanosomatideos.

RESUMO

Trypanosoma evansi é patogênico para diversas espécies de animais em áreas tropicais e subtropicais. No Brasil, a doença é endêmica no Pantanal Mato-Grossense. Este estudo analisou o perfil eletroforético das proteínas séricas de bovinos infectados experimentalmente com *T. evansi*. Foram utilizados oito bovinos, mestiços, com idade aproximada de oito meses, clinicamente sadios e soronegativos (RIFI) para *T. evansi.* Os animais foram divididos em dois grupos, G1: cinco bovinos inoculados via intravenosa com 2,2 x 10^7 tripomastigotas de *T. evansi*, e G2: três bovinos mantidos como controles. Sangue para obtenção do soro foi coletado diariamente até o 15º dia após a inoculação (DAI), e posteriormente a intervalos semanais até o 204º DAI, a cada 14 dias até o 372º DAI e a cada 30 dias até o 522º DAI. Para o fracionamento das proteínas foi utilizada a eletroforese em gel de poliacrilamida contendo dodecil sulfato de sódio (SDS-PAGE). Por meio da prova biológica, detectou-se *T. evansi* nos bovinos 01, 06 e 08 no 15º DAI, 06 e 07 no 30º DAI, 01 e 06 no 45º DAI, 06 no 60º DAI e 01 no 75º DAI. Vinte e seis proteínas com pesos moleculares variando de 20 a 245 kDa foram encontradas nos bovinos, sendo que oito foram identificadas nominalmente: imunoglobulina A (IgA), ceruloplasmina, transferrina, albumina, imunoglobulinas G (IgG) de cadeia pesada e de cadeia leve, haptoglobina e glicoproteína ácida.

PALAVRAS-CHAVE: Ruminantes. Proteína. Eletroforese. Trypanosomatideos.

[1]Departamento de Clínica e Cirurgia Veterinária, Faculdade de Ciências Agrárias e Veterinárias, UNESP. Via de Acesso Prof. Paulo Donato Castellane, s/n. CEP-14884-900 – Jaboticabal, SP. Tel. - (16) 3209 2626 - Ramal 557. *Autor para correspondência: lmarques@fcav.unesp.br

INTRODUCTION

Trypanosoma evansi is widely distributed geographically and causes diseases in animals in tropical and sub-tropical areas, especially in Africa and Latin America (LUN e DESSER, 1995). The disease is enzootic in horses from Pantanal Mato-Grossense (SILVA et al., 1995a), and has also been described in dogs, capivaras, coatis, cattle, buffalo, small marsupials and armadillos (NUNES e OSHIRO, 1990; NUNES et al., 1993; NUNES et al., 1994; SILVA et al., 1995b; HERRERA et al., 2004).

Animals infected with *T. evansi* exhibit inflammatory lesions (MARQUES, 1996; CADIOLI, 2001), and acute phase proteins increase in response to production of chemical mediators released by macrophages and leukocytes during inflammatory and infectious processes (KENT, 1992). Thus, the presence of acute phase proteins reflect the severity of the inflammatory process and may help the diagnosis and prognosis, as well as the understanding of the pathogenic mechanisms of the disease (GODSON et al., 1996).

The technique of electrophoresis on acrylamide gels containing sodium dodecyl phosphate (SDS-PAGE) is relatively simple and inexpensive, and allows to identify extremely low protein concentrations in micro amounts of sample (GORDON, 1995). Fraction changes of different serum proteins were observed in calves (VERMAN e GAUTAM, 1979), camels (BOID et al., 1980) and horses (BREM et al., 1984) infected with *T. evansi*. Sandoval et al. (1994) reported changes in the levels of total serum protein and albumin of a infected dog, while Passos (2004) observed changes in the serum levels of transferrin, haptoglobin and albumin of infected sheep. Teixeira et al. (2008) nominally identified 12 proteins in Wistar rats experimentally infected with *T. evansi* and Patelli et al. (2008) while working with goats experimentally infected with the same strain, found 21 proteins with molecular weight varying between 16 and 165 kDa, from these 6 were nominally identified (transferrin, albumin, antitrypsin, haptoglobin, acid glycoprotein and light chain IgG).

The diagnosis of trypanosomiasis is relatively easy in animals with acute infection, when parasites are present in large numbers in the peripheral blood (OLIVEIRA et al., 1989), however, it is more difficult in chronic infections when parasitemia is low and intermittent (NANTULYA, 1990; HERRERA et al., 2001). Therefore, due to the importance of trypanosomiasis and the difficulties of diagnosing the disease in ruminants, because they have low parasitemia, this study aimed to determine the serum protein levels of cattle experimentally infected with *T. evansi*.

MATERIAL AND METHODS

A total of eight crossbred cattle, seven females and one male, aged approximately 8 months old were used. Before the begin of the experiment, the animals received 200 µg/kg ivermectin based on body weight, underwent physical examination and blood tests, and were kept in screened pens in the Departamento de Clínica e Cirurgia Veterinária of FCAV, UNESP. The animals were fed freely with water, mineral salt, corn silage, Tifton hay and supplemented with feed that consisted of corn (70%) and soybean (30%), at the ratio of 2 kg/animal/day.

The animals were distributed in 2 groups: G1 - inoculated with *T. evansi* strain (animals 01, 03, 06, 07 and 08) and G2 - control (animals 02, 04 and 05). The *T. evansi* strain was isolated by Moreira and Machado (1985) from a naturally infected dog and cryopreserved at -196°C. The *T. evansi* strain was inoculated intraperitoneally into Wistar rats for replication, and G1 was infected intravenously with 2.2×10^7 trypomastigote.

The presence of trypomastigote in the peripheral blood of cattle was determined by biological test (inoculation in mice), thick blood, blood smears stained with May-Gruenwald and Strout concentration method on the 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th}, 90^{th}, 120^{th}, 150^{th}, 180^{th}, 210^{th}, 240^{th}, 270^{th}, 300^{th}, 330^{th}, 360^{th}, 390^{th}, 420^{th}, 450^{th}, 480^{th}, 510^{th} and 520^{th} days after inoculation (DAI). Serum samples were obtained from blood collected by venipuncture of the jugular immediately before inoculation, and then daily until the 15^{th} DAI, after that at weekly intervals until the 204^{th} DAI, every 14 days until the 372^{nd} DAI, and finally every 30 days until the 522^{th} DAI. Total serum protein was determined by the biuret method and protein fractionation was done by polyacrylamide gel electrophoresis containing sodium dodecyl sulfate (SDS-PAGE), according to technique described by Laemmli (1970). After fractionation, the gel was stained with coomassie blue staining for 10 minutes, followed by a 7% acetic acid solution to remove excess dye, until the protein fractions could be seen clearly. Protein concentrations were determined using a computerized densitometer (Shimadzu CS 9301, Tokyo, Japan). A marker solution (Sigma, Saint Louis, USA) with molecular weights of 36, 45, 66, 97,4, 116 and 205 kDa was used, in addition to the purified proteins haptoglobin and α1-antitrypsin.

Statistical analysis was performed using a completely randomized design for each day and the means were compared by t test.

RESULTS AND DISCUSSION

T. evansi was not detected in the peripheral blood of inoculated cattle (G1) by direct parasitological methods, possibly due to low parasitemia. The biological test, however, detected *T. evansi* in animals 01, 06 and 08 on the 15^{th} DAI, 06 and 07 on the 30^{th} DAI, 01 and 06 on the 45^{th} DAI, 06 on the 60^{th} DAI and 01 on the 75^{th} DAI. Therefore, it is possible to conclude that despite low parasitemia, the inoculated cattle harbored *T. evansi* for at least 75 days. It is noteworthy that the pre-patent period was

approximately 15 days. The mean, standard deviation and t test of the protein identified nominally in the animals of the *T. evansi* infected group (G1) and control group (G2) are presented in Tables 1 and 2.

Total protein serum levels of infected animals oscillated within the physiological limits for cattle, similar to what has been reported for horses (MARQUES, 1996) and coatis (HERRERA, 1998), even though, Kathira and Avastthi (1985) observed reduction in young buffaloes infected with the same species. These variations are probably due to different feed management, and also differences in pathogenicity between strains of *T. evansi* from different continents.

Twenty six proteins with molecular weights between 20 and 245 kDa were found in the animals inoculated with *T. evansi*, from these only eight were identified nominally (immunoglobulin A, ceruloplasmin, transferrin, albumin, heavy and light chain G immunoglobulins, haptoglobin and acid glycoprotein) while for the remainder proteins only the molecular weights were obtained. Passos (2004) suggest that the proteins that were not nominally identified are products from the degradation of the parasites, since they were not found in the animals from the control group.

Experimental studies with sheep (PASSOS, 2004), rats (TEIXEIRA et al., 2008) and goats (PATELLI et al., 2008) infected with the same *T. evansi* strain identified, respectively, 45, 31 and 21 protein fractions of molecular weights varying between 12 and 205 kDa. Therefore, the number of protein fractions of animals infected with *T. evansi* does not seem to be directly correlated with the immune response to the parasite, but possibly to the different response of each species to similar stimuli. The following acute proteins were identified, ceruloplasmin, transferrin, albumin, haptoglobin and glycoprotein. Teixeira et al. (2008) and Patelli et al. (2008) while working with Wistar rats and goats experimentally infected with the same *T. evansi* strain, identified seven and six acute phase proteins, respectively. Most acute phase proteins are glycoproteins synthesized by hepatocytes as a result of an injury, trauma or tissue infections (BAUMANN e GAULDIE, 1994).

The IgA showed significant specific reductions over the period, similar to what happened to mice (TEIXEIRA et al., 2008); however, the significance of these changes is still unknown.

Ceruloplasmin increased significantly in cattle on the 288th, 316th, 330th and 492nd DAI, period when the parasitemia was no longer detected. Teixeira et al. (2008) reported that mice also showed significant reductions in the early and intermediate stages of disease evolution, and in goats, Patelli et al. (2008) did not find this protein, on the other hand, infected sheep (PASSOS, 2004) displayed increased levels from the 5th to the 7th DAI. Similar to what happened to mice (TEIXEIRA et al., 2008) and goats (PATELLI et al., 2008) infected with *T. evansi,* transferrin, also increased significantly in cattle. However, Passos (2004) while working with sheep inoculated with the same strain, did not detect significant changes in the level of this protein. Transferrin is a negative acute phase protein whose serum levels tend to decrease in the presence of inflammation (KANEKO, 1997). Thus, it can be concluded that ceruloplasmin and transferrin levels of animals infected with *T. evansi* vary considerably with the disease, and therefore, are not reliable as a method to assist in the diagnosis of trypanosomiasis.

Albumin levels of inoculated cattle increased significantly on the 522nd DAI and decreased on the 462nd and 492nd DAI. Similarly, serum levels of this protein in animals infected with *T. evansi* changes, either increasing (PATELLI et al., 2008) or decreasing (VERMAN e GAUTAM, 1982; BREM et al., 1984; SANDOVAL et al., 1994 and TEIXEIRA et al., 2008). Relatively low albumin serum levels during the course of the experimental infection of sheep was attributed to increased globulins (PASSOS, 2004). The heavy and light chain immunoglobulins increased significantly about a year and a half after the animals were inoculated, the stage at which the parasites were no longer re-isolated, so it is inferred that these changes should not be attributed to *T. evansi.* It is known that African trypanosomiasis induces strong antibody response (LE RAY, 1975), however, the host immune response while attempting to eliminate the parasite causes tissue damage that can sometimes lead to death (NAGLE et al., 1974).

Haptoglobin and acid glycoprotein increased significantly at times during the late evolution stages. These are acute phase proteins in ruminants, which rise in the course of inflammatory and metabolic diseases and are either absent or present at very low levels in healthy animals, and are therefore valuable to determine the prognosis and to monitor these disease treatments (GANHEIM et al., 2007; GONZÁLES et al., 2007). Passos (2004) reported increasing and decreasing protein levels during several periods in sheep infected with *T. evansi.* In mice infected with *T. brucei*, haptoglobin was detected two days after inoculation and the highest level was reached on the 10th DAI (NGURE et al., 1997). From the foregoing, it is noted that changing levels of serum haptoglobin and acid protein are an important indicator of inflammatory diseases, however, during the course of the infection of cattle with *T. evansi*, the serum levels of these proteins changed at times during the late evolution stages, which allows to conclude that the inflammatory and metabolic changes caused by the parasite were insignificant.

In reality, little is known about the mechanisms by which trypanosomes survive in an immune body. The specific immune response may be related to the ability of the parasite to present antigenic variations, thus enabling the infection to persist in some animals (VICKERMAN, 1978). The protective role of antibodies is much discussed, but there is evidence that they help to limit the infection (BRENER e ANDRADE, 1979). In the light of current knowledge, it is known that the existing immunity during the chronic phase is not total or absolute (BOERO, 1974),

Table 1 - Means (M), standard deviations (SD) and t test (0 DAI to 176 DAI) results for serum total protein and protein fractions nominally identified by trace acrylamide gel electrophoresis (SDS-PAGE), of cattle inoculated with *T. evansi* (G1) and those of the control group (G2).

			Days after inoculation														
Proteins			0	1	2	3	4	5	6	7	14	29	57	85	120	148	176
Total	G1	M	7.04	6.90	6.54	6.60	6.61	6.75	6.69	6.51	6.74	6.90	6.94	7.01	7.52	6.84	7.18
		SD	0.34	0.44	0.12	0.29	0.54	0.38	0.39	0.38	0.40	0.41	0.26	0.58	0.27	0.31	0.33
	G2	M	6.82	7.02	6.71	6.57	6.66	6.80	6.71	6.45	6.79	6.69	7.00	6.98	7.50	7.05	7.17
		SD	0.38	0.26	0.21	0.29	0.10	0.32	0.33	0.16	0.57	0.42	0.44	0.40	0.79	0.45	0.50
	Test	t	0.42 ns	0.69 ns	0.20 ns	0.89 ns	0.87 ns	0.86 ns	0.95 ns	0.80 ns	0.88 ns	0.51 ns	0.79 ns	0.94 ns	0.94 ns	0.45 ns	0.96 ns
IgA 180	G1	M	107.93	115.26	106.84	105.15	129.34	129.05	147.30	104.07	121.00	107.81	106.59	83.76	127.06	160.82	117.86
		SD	28.82	27.71	37.38	31.94	30.95	31.76	41.69	26.12	74.63	39.96	78.86	54.54	36.88	56.11	39.25
	G2	M	165.48	154.17	81.66	104.09	152.11	212.21	208.55	166.68	141.19	79.82	119.61	105.78	178.26	159.74	136.19
		SD	15.99	3.43	33.90	50.15	25.98	21.43	22.79	15.86	6.43	20.73	55.57	47.22	45.55	13.29	59.69
	Test	t	0.02*	0.06 ns	0.38 ns	0.97 ns	0.33 ns	0.01**	0.06 ns	0.01*	0.67 ns	0.31 ns	0.81 ns	0.58 ns	0.13 ns	0.98 ns	0.61 ns
Ceruloplasmin 128	G1	M	42.05	49.93	39.24	31.62	30.51	40.75	39.54	47.54	55.08	46.86	47.11	30.29	29.94	40.01	25.69
		SD	12.17	20.59	9.08	14.79	10.99	14.59	12.26	13.41	17.29	18.23	20.71	17.97	11.44	13.69	4.99
	G2	M	42.46	34.36	37.29	26.16	21.91	22.84	48.15	54.15	59.96	34.11	32.63	20.99	20.01	34.23	24.13
		SD	4.42	9.32	14.66	3.06	6.56	5.71	30.18	26.51	20.80	5.16	9.66	6.40	6.46	9.98	11.00
	Test	t	0.96 ns	0.27 ns	0.82 ns	0.56 ns	0.27 ns	0.09 ns	0.58 ns	0.65 ns	0.73 ns	0.29 ns	0.31 ns	0.43 ns	0.23 ns	0.55 ns	0.79 ns
Transferrin 82	G1	M	249.91	251.01	243.89	262.45	218.28	956.20	222.38	232.85	278.63	331.55	328.13	261.60	337.70	1.164.38	248.14
		SD	32.95	32.57	21.91	45.64	77.17	1.599.82	34.18	26.06	23.96	30.44	20.70	25.39	80.33	1.865.08	15.78
	G2	M	282.06	279.22	263.34	323.43	228.10	270.38	242.38	256.79	302.58	360.69	380.45	269.56	381.32	383.09	264.26
		SD	24.20	10.83	18.07	95.22	32.11	48.39	78.74	32.62	23.94	32.26	20.10	48.17	38.87	20.47	13.31
	Test	t	0.20 ns	0.21 ns	0.25 ns	0.26 ns	0.84 ns	0.50 ns	0.63 ns	0.29 ns	0.22 ns	0.25 ns	0.01*	0.76 ns	0.42 ns	0.51 ns	0.19 ns
Albumin 66	G1	M	4294.25	4319.27	3773.45	4121.85	3658.01	3306.55	3720.10	4041.41	3858.61	3755.83	3883.39	4073.73	4795.17	3415.13	4362.05
		SD	285.65	207.20	238.32	297.45	325.74	1.002.16	190.86	270.63	407.60	318.65	207.06	250.19	218.65	1.434.91	269.01
	G2	M	4103.05	4303.27	3842.19	3854.30	3746.08	3767.43	3688.68	3857.89	4024.86	3674.81	3931.11	4310.20	5030.30	4215.93	4605.58
		SD	246.31	346.28	372.70	393.61	112.90	300.71	115.12	153.22	210.70	354.01	163.60	241.37	480.53	253.00	255.40
	Test	t	0.37 ns	0.94 ns	0.76 ns	0.31 ns	0.68 ns	0.48 ns	0.81 ns	0.33 ns	0.54 ns	0.75 ns	0.75 ns	0.24 ns	0.37 ns	0.39 ns	0.25 ns
Heavy chain IgG 57	G1	M	1.195.81	1.181.81	1.249.39	981.63	1.199.46	1.053.30	1.321.31	1.108.56	1.192.25	1.230.06	1.386.27	1.123.94	891.19	923.33	1.068.31
		SD	156.22	191.43	169.95	235.83	208.97	615.65	236.94	221.09	177.63	248.57	267.38	189.31	425.95	600.66	242.54
	G2	M	1143.90	1197.96	1270.85	1103.95	1244.62	1317.42	1239.14	1042.84	1152.45	1156.17	1272.98	1068.99	879.91	1061.04	879.26
		SD	168.09	145.12	126.61	116.00	201.24	239.34	244.08	117.79	331.42	106.95	110.34	26.90	211.05	160.28	145.53
	Test	t	0.67 ns	0.90 ns	0.86 ns	0.44 ns	0.77 ns	0.51 ns	0.65 ns	0.66 ns	0.83 ns	0.65 ns	0.52 ns	0.65 ns	0.97 ns	0.72 ns	0.27 ns
Haptoglobin 42	G1	M	8.39	10.02	21.66	17.62	27.60	18.93	33.20	11.49	14.54	17.29	22.88	13.96	146.76	26.85	15.37
		SD	5.35	5.60	3.17	6.98	18.90	8.74	10.94	8.28	10.35	1.61	9.53	8.85	285.67	13.87	10.74
	G2	M	6.98	14.00	24.42	19.59	19.04	22.64	32.21	9.67	13.59	17.21	24.68	17.64	9.74	26.03	15.69
		SD	2.82	9.25	16.25	3.11	5.57	8.89	6.45	3.00	6.39	8.72	10.99	11.07	5.45	3.12	6.43
	Test	t	0.69 ns	0.47 ns	0.71 ns	0.67 ns	0.49 ns	0.58 ns	0.89 ns	0.73 ns	0.89 ns	0.98 ns	0.81 ns	0.62 ns	0.45 ns	0.93 ns	0.97 ns

Acid glycoprotein 38	G1	M	8.87	7.40	15.26	18.67	22.00	11.00	16.73	8.90	10.64	15.46	14.88	12.87	17.68	15.47	14.88
		SD	4.90	2.02	9.95	7.82	23.28	5.61	7.72	3.72	5.16	5.38	3.79	3.92	9.21	6.87	7.51
	G2	M	6.28	6.32	11.69	11.89	14.65	8.92	24.74	7.45	8.50	12.88	15.36	15.98	11.35	22.72	21.84
		SD	0.75	5.47	1.48	4.08	8.22	4.66	19.21	1.93	1.08	4.15	9.27	1.79	4.19	1.71	3.20
	Test	t	0.41 ns	0.69 ns	0.57 ns	0.22 ns	0.63 ns	0.61 ns	0.42 ns	0.56 ns	0.52 ns	0.51 ns	0.92 ns	0.25 ns	0.31 ns	0.13 ns	0.19 ns
Light chain IgG 26	G1	M	488.08	452.19	587.82	513.22	731.98	559.92	748.86	495.66	526.13	647.40	601.64	661.60	476.69	502.45	549.02
		SD	85.95	132.75	116.14	232.22	154.56	274.23	201.42	141.38	156.80	152.28	182.64	153.08	227.23	371.70	122.03
	G2	M	384.90	220.45	603.77	558.15	660.40	481.17	491.88	483.69	485.12	695.19	575.24	581.18	510.37	431.68	540.25
		SD	217.61	248.72	48.93	71.30	199.00	363.48	428.03	8.91	127.91	159.23	107.38	101.28	18.45	77.88	105.95
	Test	t	0.36 ns	0.13 ns	0.83 ns	0.76 ns	0.59 ns	0.74 ns	0.28 ns	0.89 ns	0.72 ns	0.69 ns	0.83 ns	0.46 ns	0.81 ns	0.76 ns	0.92 ns

Table 2 - Means (M), standard deviations (SD) and t test (206 DAI to 522 DAI) results for serum total protein and protein fractions nominally identified by trace acrylamide gel electrophoresis (SDS-PAGE), of cattle inoculated with *T. evansi* (G1) and those of the control group (G2).

PM (KD) Protein			**Days after inoculation**														
			206	232	246	260	274	288	302	316	330	358	402	432	462	492	522
PT	G1	M	7.07	7.20	7.66	7.77	8.08	7.58	7.78	7.54	7.52	7.46	7.61	7.41	7.45	7.02	8.15
		SD	0.55	0.39	0.62	0.14	0.34	0.28	0.35	1.16	0.39	0.34	0.57	0.32	0.35	0.37	0.49
	G2	M	7.12	4.31	6.98	6.95	7.07	7.01	7.17	6.72	6.83	6.69	6.98	7.17	6.78	7.00	7.45
		SD	0.53	3.76	0.28	0.53	0.61	0.53	0.50	0.53	0.30	0.32	0.28	0.15	0.44	0.44	0.54
	Test	t	0.92 ns	0.12 ns	0.13 ns	0.01*	0.02*	0.09 ns	0.09 ns	0.30 ns	0.04*	0.02*	0.13 ns	0.27 ns	0.05 ns	0.96 ns	0.11 ns
IgA 180	G1	M	70.35	109.83	252.80	181.56	128.05	146.92	95.83	127.20	107.87	190.65	137.43	116.68	112.24	97.09	150.66
		SD	42.04	14.77	130.05	102.00	79.85	24.13	55.99	50.73	64.72	99.85	32.66	69.43	23.54	44.95	10.02
	G2	M	61.92	88.47	159.33	144.84	85.24	120.38	107.66	159.69	155.05	116.59	60.62	96.63	210.09	61.04	209.62
		SD	18.55	59.97	50.19	13.71	27.14	58.47	44.50	38.44	16.99	75.98	17.53	65.39	77.54	18.86	67.15
	Test	t	0.76 ns	0.46 ns	0.29 ns	0.57 ns	0.42 ns	0.39 ns	0.77 ns	0.38 ns	0.27 ns	0.32 ns	0.01*	0.70 ns	0.03*	0.24 ns	0.09 ns
Ceruloplasmin 128	G1	M	30.90	34.22	54.93	62.74	58.11	62.27	69.71	61.97	69.55	60.55	49.60	61.18	67.26	43.64	84.54
		SD	1.70	7.14	22.29	18.82	30.14	6.76	17.25	13.36	13.20	15.11	10.12	7.42	46.45	8.71	25.79
	G2	M	23.49	23.72	50.54	60.68	36.28	32.75	21.67	17.89	33.14	33.87	22.91	25.41	53.61	23.16	68.58
		SD	13.19	4.71	13.54	17.76	7.60	10.27	7.08	5.36	9.43	25.43	12.77	4.21	7.80	13.28	10.43
	Test	t	0.24 ns	0.07 ns	0.77 ns	0.88 ns	0.28 ns	0.00**	0.00**	0.00**	0.01*	0.11 ns	0.02	0.00**	0.64 ns	0.04*	0.36 ns
Transferrin 82	G1	M	248.81	244.29	337.35	368.30	243.11	231.05	1.710.94	177.97	241.70	220.99	197.23	1.026.81	188.98	182.24	213.68
		SD	32.04	30.53	55.93	50.36	48.32	30.36	2.070.57	57.39	20.35	23.21	39.05	2.015.53	60.35	38.26	100.69
	G2	M	264.71	262.85	316.43	309.62	382.72	380.37	276.56	342.41	371.01	1.628.52	259.37	282.48	169.13	260.37	225.17
		SD	29.83	13.18	34.50	23.50	55.89	12.51	48.32	32.27	11.39	2.379.46	19.89	7.07	26.18	25.53	46.91
	Test	t	0.51 ns	0.37 ns	0.59 ns	0.11 ns	0.01**	0.00**	0.29 ns	0.00**	0.00**	0.21 ns	0.05 ns	0.56 ns	0.62 ns	0.02*	0.86 ns

Albumin 66	G1	M	4540.07	4291.64	4277.39	4342.95	4349.87	4398.40	3167.53	4418.81	4129.23	4273.54	3937.26	3916.84	4588.73	3618.65	5107.52
		SD	228.03	302.02	382.41	157.62	358.13	354.15	1.584.57	807.28	180.70	161.27	446.82	1.598.38	248.73	158.24	238.20
	G2	M	4610.48	4087.16	4156.05	4128.14	3894.09	3935.34	4423.60	4512.12	4083.43	3102.00	4521.26	4381.57	3977.54	4535.28	4532.23
		SD	364.21	434.12	208.84	399.51	531.33	245.81	257.34	314.92	188.64	2.005.53	180.46	195.13	359.03	257.91	162.13
	Test	t	0.74 ns	0.46 ns	0.64 ns	0.31 ns	0.19 ns	0.10 ns	0.23 ns	0.86 ns	0.74 ns	0.22 ns	0.08 ns	0.64 ns	0.03*	0.00**	0.01*
Heavy chain IgG 57	G1	M	932.09	1131.28	1345.28	1395.53	1535.58	1352.75	927.14	1279.54	1463.09	1371.38	1250.44	929.94	1003.91	1156.20	1171.06
		SD	259.30	318.03	264.94	109.71	193.10	201.20	881.30	72.34	302.46	242.70	169.31	534.03	222.34	176.13	157.56
	G2	M	780.76	994.87	1068.51	1171.41	1218.96	1272.99	1097.66	787.82	1026.00	559.04	765.62	1066.85	1099.59	768.96	1216.94
		SD	103.65	139.32	182.35	284.20	53.51	100.69	54.45	174.65	133.51	491.40	89.09	111.78	21.86	105.70	270.86
	Test	t	0.38 ns	0.52 ns	0.17 ns	0.15 ns	0.04*	0.55 ns	0.76 ns	0.00**	0.06 ns	0.02*	0.00**	0.69 ns	0.50 ns	0.01*	0.77 ns
Haptoglobin 42	G1	M	16.57	16.21	17.40	24.93	28.06	16.06	31.62	14.87	24.43	27.86	43.96	15.34	20.53	59.41	13.11
		SD	5.06	8.69	5.93	11.61	24.04	11.55	23.08	5.44	12.15	8.06	14.33	11.25	11.39	40.67	4.66
	G2	M	15.95	23.58	15.38	13.69	17.96	24.73	19.37	8.71	25.19	15.59	15.59	25.68	15.71	15.62	17.18
		SD	5.16	5.07	3.43	5.72	8.78	10.99	9.79	4.70	2.49	7.10	4.62	7.49	4.76	4.64	5.96
	Test	t	0.87 ns	0.24 ns	0.61 ns	0.18 ns	0.52 ns	0.34 ns	0.43 ns	0.16 ns	0.92 ns	0.07 ns	0.02*	0.21 ns	0.52 ns	0.12 ns	0.32 ns
Acid glycoprotein 38	G1	M	12.25	12.45	17.15	19.95	34.48	10.00	35.50	16.22	23.50	23.93	51.11	29.72	32.11	44.08	49.16
		SD	2.98	3.74	7.95	17.38	43.40	5.30	28.63	12.33	5.72	10.32	6.09	20.10	13.84	4.17	20.11
	G2	M	16.66	16.64	17.25	8.67	13.46	15.32	20.61	10.26	22.00	14.15	16.35	17.98	41.54	16.44	37.64
		SD	3.66	1.43	2.19	0.71	3.70	9.11	5.36	4.02	1.09	9.93	3.51	2.61	19.37	3.83	17.64
	Test	t	0.11 ns	0.12 ns	0.99 ns	0.32 ns	0.45 ns	0.33 ns	0.42 ns	0.46 ns	0.68 ns	0.24 ns	0.00**	0.37 ns	0.45 ns	0.00**	0.45 ns
Light chain IgG 26	G1	M	648.26	545.02	538.23	621.71	785.84	651.93	672.96	725.05	474.72	479.60	731.07	417.83	541.56	450.64	413.18
		SD	150.08	311.50	106.30	125.49	302.65	79.85	206.30	286.29	146.88	137.44	416.14	262.86	121.81	364.03	246.67
	G2	M	623.91	520.84	530.97	492.96	729.27	575.08	597.57	458.32	274.28	470.69	439.19	556.12	516.26	445.98	613.68
		SD	84.03	95.78	70.92	105.00	128.94	103.07	114.18	6.65	247.14	147.14	354.88	67.38	93.54	362.03	121.83
	Test	t	0.81 ns	0.90 ns	0.92 ns	0.19 ns	0.77 ns	0.28 ns	0.59 ns	0.17 ns	0.19 ns	0.93 ns	0.35 ns	0.42 ns	0.77 ns	0.99 ns	0.25 ns

however, it is assumed that antibodies help to control parasitemia caused by *T. evansi*.

CONCLUSION

The results presented showed no pattern in the increase or decrease of plasma proteins in cattle experimentally infected with the cryopreserved strain of *T. evansi.* Thus, the electrophoretic fractionating of proteins from the acute phase can not be used as a helping tool to diagnose trypanosomiasis in cattle.

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