THE AVIRULENCE OF THE CULTIVATED PF STRAIN OF TRYPANOSOMA CRUZI. VI — THE EFFECT OF ANTILYMPHOCYTIC SERUM IN DOGS.

Humberto Menezes *

The use of antilymphocytic serum (ALS) in dogs suppressed the immune response of a vaccinated and simultaneously ALS treated animal, increased the infectivity of a virulent strain and did not induce a typical infection-disease in 3 dogs receiving the serum and the avirulent PF strain.

INTRODUCTION

The antilymphocytic serum (ALS) in the only substance that really can be considered as an immunosupressive agent (Medawar[12]). The impairment of the immune response in animals treated with ALS and infected with *P. berghei* and *T. rhodesiense* (Walker[22]), *T. brucei* and *T. congolense* (Luckins[11]), *BCG and Leishmania enriettii* (Bryceson et al.[5]) have been already rescribed.

The enhancement of experimental *trypanosomiasis cruzi* in animals, with immunosupressive drugs was demonstrated by Thierman et al.[20], Camargo et als.[6], Kumar et als.[9] Brener et al.[3] and Menezes[17].

Aiming to confirm the avirulence of the *T. cruzi* long term cultivated *Y* strain, that we designated *PF* strain, we depressed the immune response of mice with Prednisolone (Menezes[15,16]) and antiproliferative agents (Menezes[17]).

In a previous paper we have demonstrated the immunogenic activity of the *PF* strain to dogs (Menezes[13]) and we intend to stablish now that the aforesaid avirulence is also present in dogs submitted to a powerfull immunosuppressive agent-the ALS.

MATERIAL AND METHODS

A — *Vaccines.* — The liquid phase of three tubes with 15 days old culture of the *PF* strain, in Packchanian medium, was centrifuged and the sediment washed several times in saline solution. The final suspension had almost 10^8 parasites per ml, with 85% of mobile forms and about 3% of trypomastigotes. Another vaccine was prepared by a similar manner, having almost 70% of mobile flagellates and a concentration of $2{,}9 \times 10^6$ parasites per ml with more or less 3% of trypomastigotes. Both suspension were used immediatly after preparation and will be designated as *vaccine*.

B — *Animals.* — Five litter-mate puppies of a common mixed race were employed in this experiment and belonged to the group

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named *Fr 59A*. The age of the animals, at the begining of the experience was 4 months and the mean body weight 4,0 kg.

The dog Fr 59-2 pertained to another group and was months old, weighting 0,95 kg at the start of the experiment.

C — *Antilymphocytic serum* (ALS) — The serum was prepared following the technique described by Abaza et als.[1], from thoracic duct lymphocytes of dogs, injected in horse. The ALS was a purifiel globulin with the leuco-aglutination titer of 1/512.

All the examinations done in the animals, previously and/or during the experiment, are shown in Tables.

The dogs submited to the ALS treatment received, every two days, an intramuscular injection of 200.000 IU of Penicillin-Procaine. **.

The virulent infections were done by intraperitoneal injections of blood forms of the virulent *Y* strain, obtained from mice at the 8th day of infection.

Gross and histological examination was performed on several organs of all the animals.

RESULTS AND COMMENTS

It can be concluded from the observation of the Graph I and Tables 1-3 that the dogs vaccinated with the *PF* strain of the *Trypanosoma cruzi* had neither positive parasitoscopy nor mortality during the nine months duration of the experiment.

From the 3 vaccinated and ALS treated dogs, the first presented all (except one) serological and parasitological tests negative (Table 1). The animal had no inflamatory infiltration in the myocardium (Fig. 1), but had microabcess in the liver (Fig. 2), lesion surely not related to *Trypanosoma cruzi* infection.

The second dog has shown 3 positive blood cultures, being the last 3 negative. Two blood inoculations in mice were positive too, but the last 4 were negative. All the xenodiagnosis (10) gave negative results. The CFT (Guerreiro & Machado test) became positive since the first positive hemoculture and remained as such till the end of the experiment (Table 2).

In the liver, an infiltration of the trials by neutrophiles seems due to an ascendent pyogenic infection.

No parasites could be detected in the several histologic sections examined (Fig. 9 & 12).

The third vaccinated dog had only 1 positive blood culture out of 9, and the last 3 were negative. The CFT, with the same blood sample, gave discordant results in 2 different laboratories (Table 3). The other parasitological tests were negative and the ECG presented no abnormalities.

No macroscopic alterations were seen. The histophatological examination demonstrated the presence of granulomatous lesions in the myocardium (Figs. 5-6) and in the liver (Figs. 3-4). The lesions were similar in both organs and composed of histiocytes envolved by eosinophiles and lymphocytes. An outline of giant cell appeared in the cardiac lesion (Fig. 6). No parasites were seen and we never found a such lesion in American trypanosomisis. It was caused, probably, by another parasite (granuloma forming), stimulated by the immunesuppression.

After a high dose of a potent ALS and simultaneous vaccination with a live avirulent strain of *T. cruzi*, all the 3 dogs remained alive with normal ECG. In spite of the transient positive blood cultures and blood inoculations in two dogs and the persistent positive CFT in one of them and oscilating in another, the result of the experiment must be considered as very good, since we know from the literature that the simultaneous use of live (virulent or low virulent) parasites and immunosupressive agents induces high parasitemia, severe damage and frequently the death of the experimental animals (Camargo et als.[6]; Menezes[17]; Bryceson et al.[5]; Walker[22]; Thierman et al.[20] and Vilches et al.[21]).

The aggravating role of the ALS could be observed with the animal Fr 59A-4 (Table 4, Graph I, Fig. 10).

Very interesting was the case of the dog Fr 59-2 (Table 6) vaccinated and simultaneously treated with ALS.

As expected, the immune response of this animal was blocked and when, 30 days later, he was challenged, became infected and died of an acute trypanosomiasis, with

** Benzetacil — Fontoura-Wyeth

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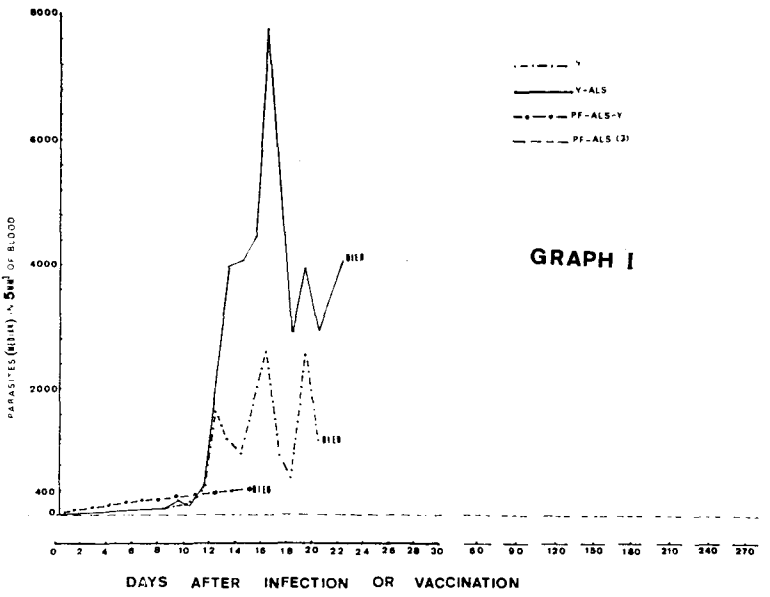

the ECG presenting AV block (Table 6), pseudo-cysts and inflamatory reaction in the bundle of His (Fig. 11) and positive parasitemia (Table 6).

The activity of the ALS was detected by the enhancement of the Y infection and also by the histologic alterations observed in the lymphoid organs of the treated dogs (Figs. 7-8). The high absolute counting of lymphocytes in ALS treated animals does not mean weakness in the activity of the serum, since it is well known that no close correlation exists between the number of lymphocytes, in the peripheral blood, and the immunosuppressive effect of that agent (Denman et als.[7]; Levey et al.[10] and Argyris et al. [2]). In the lymphoyd tissue of the ALS treated animals almost all the small lymphocytes were substituted by large proliferative cells (Fig. 8), as seen by Iwasaki et als.[8] and Woodruff et al.[23].

To those interested in the mechanism of action of the ALS we recomend a review of Mitchison.[18].

It seems that, in the present experiment, blood culture was the best technique for the detection of *T. cruzi* in the blood stream, surpassing the blood inoculation and the xenodiagnosis.

CONCLUSIONS

Antilymphocytic serum was able to enhances a virulent *T. cruzi* infection in dogs that died 21 days after the infection, but was unable to promote evident infection-disease in animals injected with a live avirulent strain (PF) of the same parasite and killed nine months later.

Animal injected with the avirulent *PF* strain and simultaneously treated with ALS does not develop immunity against a further virulent infection, dying from an acute trypanosomiasis.

Acknowledgments — Several people helped me in doing this work.
To all of them my deepest appreciation.

RESUMO

Soro antilinfocitário ALS anti-cão mostrou-se capaz de agravar uma infecção produzida pela cepa virulenta Y do Trypanosoma cruzi *(Graph I, Table 4).*

Cães vacinados com a cepa avirulenta PF *não demonstraram, 9 meses após o início da experiência, qualquer sinal de infecção-doença, apesar das altas doses de soro recebidas (Graph I, Tables 1-3).*

O soro é um poderoso imunosupressor capaz de impedir o desenvolvimento de defesas imunitárias em um animal injetado com a cepa PF *e simultaneamente tratado com o mesmo (Table 6).*

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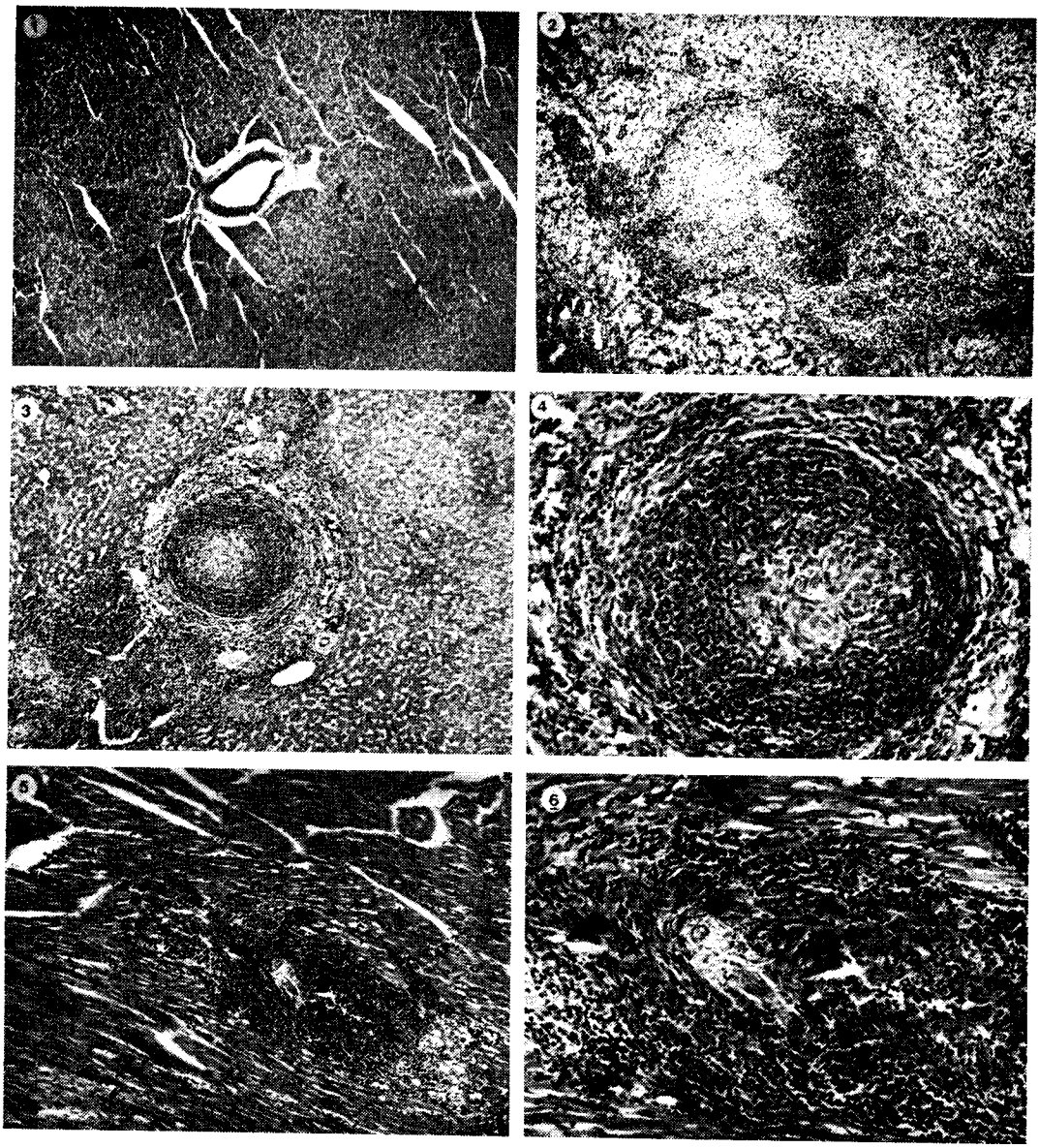
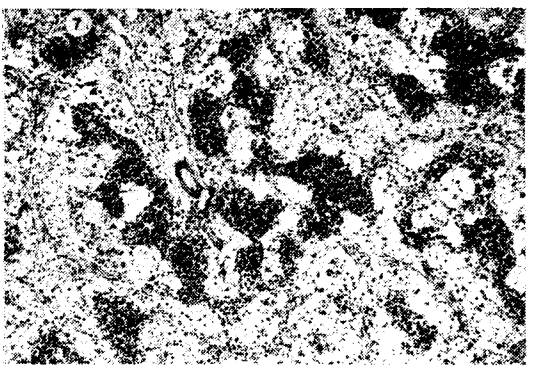
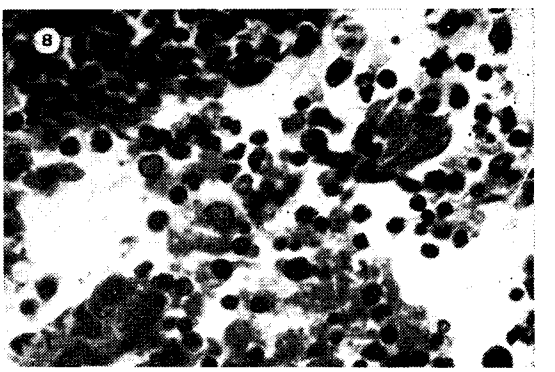
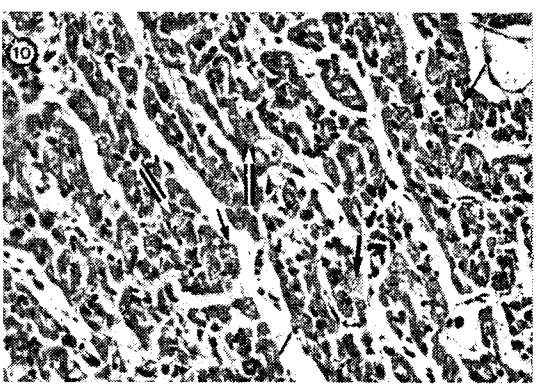
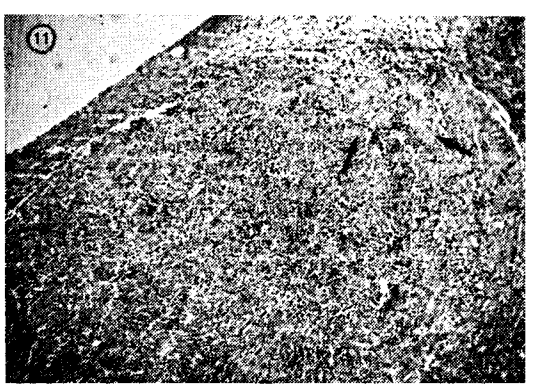


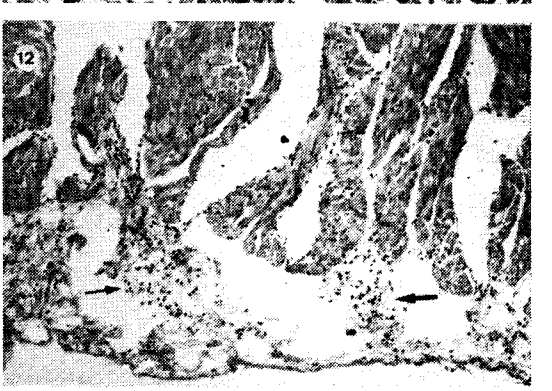
1 — Normal myocardium. Animal Fr 59A-1 (Vaccine+ALS). Orig. 25X
2 — Hepatic microabcess in the dog Fr 59A-1 (Vaccine+ALS). Orig. 25X
3 — Granulomatous lesion in a portal trial. Animal Fr 59A-3 (Vaccine+ALS). Orig. 25X
4 — Idem. Orig. 64X
5 — Granulomatous lesion in the myocardium. Dog Fr 59A-3 (Vaccine+ALS). Orig. 25X
6 — Idem. Orig. 64X











7 — Lymphnode of an ALS treated dog (Fr 59A-4) showing the scarcity of lymphocytes in the sinuses. Orig. 25X

8 — Lymphnode sinus of the same animal with large lymphoid cells. Orig. 160X

9 — Very small inflamatory infiltration, without parasites, in the muscular layers of the oesophagus of the dog Fr 59A-2 (Vaccine+ALS). Orig. 25X

10 — Miocardium of the dog Fr 59A-4 infected with the virulent Y strain and treated with ALS. Almost all the myofibers have pseudo-cysts (→). No inflamatory infiltration. Orig. 25X

11 — Myocardium (IVS) of the animal Fr 59-2 (Vaccine+ALS+Infection) Showing pseudo-cysts and inflamation at the level of the His bundle. Orig. 25X

12 — Scant inflamatory reaction in the auricular epicardium of the dog Fr 59A-2 (Vaccine+ALS). Orig. 25X

TABLE 1

Dog FR5 9A-1 vaccinated and treated with ALS.

Day	Body wt kg	Lymph. mm³	VAC. 1 ml.	ALS. ml.	ECG	* Xeno	** Hemoc.	*** G A — M B	**** Inoc.	***** Parasit.	Obs.
0	3,0	3.382				—		— —			
1	4,0	2.354		4,0							
2	3,8	2.354									
3	3,8			3,8							
4	4,0	1.443	10^8								
5	3,8			3,8							
7	3,9			3,9							
8		3.570									
9	4,7			4,7					—	—	
11	4,7	3.132		4,7	N	—	—				
13	4,7			4,7							
14		980		4,7							
15	4,7			2,4							
17	5,3			2,4							
19	5,4			2,7		—	—			—	
21	5,4			2,7	N						
23				2,7		—		— —			
24		2.400									
25	5,4			2,7					—		
27	5,4			2,7					—		
29	5,4			2,7					—	—	
30		2.884									
31	6,0			3,0							
33	6,0			3,0	N	—	—	— —	—	—	
35	6,5	3.300			N	—	—	— —	—	—	
63	9,0				N	—	—	— —	—	—	
93	12,0				N	—	—	— —	—	—	
123	14,0				N	—	—	— 1,9	—	—	
153	17,0				N	—	—	— —	—	—	
183	16,0				N	—	—	— —	—	—	Killed
243								— —			
273	20,0										

* 5 nymphes *R. prolixus*, 5th instar. each test.
** 3 tubes Warren medium, each test.
*** Guerreiro & Machado (CFT) — Quantitative test (Waldsworth, Maltner & Maltner). A and B dif. labs. — Positive $>$ 1,9.
**** 5 mice 10 g, each test.
***** Parasites peripheral blood. Strout's technique (19).

TABLE 2

Dog FR5 9A-2 vaccinated and treated with ALS.

Day	Body wt kg	Lymph. 1 mm³	VAC. 1 ml.	ALS. ml.	ECG	* Xeno	** Hemoc.	*** G & M A B	**** Inoc.	***** Parasit.	Obs.
0	2,5	3.990			N	—		— —			
1	3,5			3,5							
2		2.436									
3	3,3			3,3							
4	3,4	1.414	10^8								
5	3,4			3,4							
7	3,4			3,4							
8		1.220									
9	3,8			3,8							
11	3,8	1.110		3,8	N	—	—	— —	—	—	
13	3,8			3,8							
14		1.134									
15	3,8			1,9							
17	3,8			1,9						—	
19	4,4			2,2		—	—				
21	4,3			2,2	N	—		— —			
23	4,4			2,2							
24		1.848									
25	4,3			2,2							
27	4,3			2,2							
29	4,3			2,2							
30		2.618									
31	4,8			2,4							
33	5,0			2,5							
35	5,0	1.526			N	—	—	— —	—	—	
63	7,0				N	—	+	> 2,6 > 3,0	+	—	
93	7,8				N	—	+	> 2,8 > 3,0	+	—	
123	10,5				N	—	+	> 2,8 > 3,0	—	—	
153	12,0				N	—	—	> 2,8 > 3.0	—	—	
183	12,9				N	—	—	> 2,2 > 2,7	—	—	
243	13,2				N	—	—	> 3,0 > 2,5	—	—	
273	13,8							> 3,0 > 3,0			Killed

* 5 nymphes *R. prolixus*, 5*th* instar. each test.
** 3 tubes Warren medium, each test.
*** Guerreiro & Machado (CFT) — Quantitative test (Waldsworth, Maltner & Maltner). A — B dif. labs. Positive > 1,9.
**** 5 mice 10 g, each test.
***** Parasites peripheral blood. Strout's technique (19).

TABLE 3

Dog FR5 9A-3 vaccinated and treated with ALS.

Day	Body wt kg	Lymph. mm³	VAC. 1 ml.	ALS. ml.	ECG	Xeno *	Hemoc. **	G & M *** A B	Inoc. ****	Parasit. *****	Obs.
0	2,7	6.076						— —		—	
1	3,7				N	—					
3		2.332		3,7							
2	3,7										
4	3,9	1.534		3,7							
5	3,9		10^8								
7	3,7			3,9							
8		2.346		3,7							
9	4,0										
11	4,0	1.224		4,0				— —	—	—	
13	4,0			4,0	N	—	—				
14		1.152		4,0							
15	4,0										
17	4,0			2,0							
19	4,0			2,0							
21	4,4			2,0		—	—	— —	—	—	
23	4,4			2,2	N	—					
24		1.110		2,2							
25	4,5										
27	4,4			2,3							
29	4,4			2,2							
30		1.674		2,2							
31	4,8										
33	5,0			2,4				1,5 —	—	—	
35	5,2	3.000		2,5	N	—	—	—	—	—	
63	7,9				N	—	—	— > 3,0	—	—	
93	8,5				N	—	—	> 2,5 > 2,3	—	—	
123	12,0				N	—	+	> 3,0 > 3,0	—	—	
153	12,0				N	—	—	— 1,9	—	—	
183	11,2				N	—	—	—	—	—	
243	12,8					—	—	1,6 —			
273	13,2							1,9 > 3,0			Killed

* 5 nymphes *R. prolixus*, 5*th* instar. each test.
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TABLE 4

Dog FR5 9A-4 infected with the virulent Y strain and treated with ALS.

Day	Body wt kg	Lympho-cytes mm³	INFEC. p/g bw	ALS. ml.	ECG	Xeno *	Hemoc. **	G A & M B ***	Inoc. ****	Parasit. *****	Obs.
0	3,7	2.430			N	—		— —			
1	5,0			5,0							
2		1.488									
3	4,8			4,8							
4	5,0	1.008	4.000								
5	5,0			5,0							
7	5,1	2.700		5,1							
9	5,5			5,5							
11	5,7	534		5,7	N	—	+	— —	+	—	
12										70p/5mm³	
13	5,7			5,7						175p/5mm³	
14		2.492								105p/5mm³	
15	5,7			2,9						420p/5mm³	
16	5,7									2450p/5mm³	
17	5,7			2,9						3955p/5mm³	
18	6,2									4025p/5mm³	
19	6,4			3,2						4375p/5mm³	
20										7700p/5mm³	
21	6,4			3,2	N			— 2,5		3675p/5mm³	
22										2890p/5mm³	
23	6,4			3,2						3870p/5mm³	
24		5.600								2940p/5mm³	
25	6,5			3,3						4025p/5mm³	
26	6,4										DIED

* 5 nymphes *R. prolixus*, 5*th* instar. each test.
** 3 tubes Warren medium, each test.
*** Guerreiro & Machado (CFT) — Quantitative test (Waldsworth, Maltner & Maltner). A and B dif. labs. — Positive > 1,9.
**** 5 mice 10 g, each test.
***** Parasites counting: Pizzi & Brener Technique (4).

TABLE 5

Dog FR5 9A-5 infected with the virulent Y strain

Day	Body wt kg	Infec. p/gBW	ECG	* Xeno	** Hemoc.	G A & M B ***	**** Inoculation	***** Parasitemia	Obs.
0	2,6		N	—		— —			
1	4,0	4.000							
3	3,9								
4	4,0								
7	3,9		N	+	+	— —	—		
8								105p/5mm³	
9								140p/5mm³	
10								140p/5mm³	
11	4,0							280p/5mm³	
12								1225p/5mm³	
13								1155p/5mm³	
14	4,1							980p/5mm³	
15								1505p/5mm³	
16								2590p/5mm³	
17			*Ab*			— 2,7		945p/5mm³	
18								525p/5mm³	
19								2540p/5mm³	
20								1190p/5mm³	
21	4,3								DIED

* 5 nymphes *R. prolixus*, 5*th* instar, each test.
** 3 tubes Warren medium, each test.
*** Guerreiro & Machado (CFT) — Quantitative test (Waldsworth, Maltner & Maltner). A — B dif. labs. Positive $>$ 1,9.
**** 5 mice 10g, each test.
***** Parasites counting: Pizzi & Brener Technique (4).
Ab — T wave inversion (V_6).

TABLE 6

Dog FR59 — 2 vaccinated, treated with ALS and infected with virulent Y strain.

Day	Body wt kg	Lympho-cytes mm³	VAC. INF.	ALS. ml.	ECG	* Xeno	** Hemoc.	G A	&	*** M B	**** Inoc.	***** Parasit.	Obs.
0	0,80	2.767				—		—		—			
1	0,95			1,0									
2		2.784		0,5									
3	1,5	814		1,5									
4		2.992		1,2									
5				1,2									
6		1.512	VAC(A)										
7	1,5	2.716		2,0								—	
8	1,3	3.060		2,0								—	
9		1.690		2,0								—	
10		2.070		2,0								—	
11		3.520		2,0								—	
12		2.538		2,0								—	
13		1.770		2,0								—	
14		2.240		2,0		—	—				—	—	
15		2.660		2,0								—	
16		2.376		2,0								—	
17		2.376		2,0									
18		4.896		2,0									
19				1,0								—	
20													
21	1,5	1.904			N	—	—	1,9			—	—	
24	1,3												
25		3.350											
30	1,4												
35	1,5	1.728						1,5					
43	1,7		INF(B)									280p/5mm³	
50	1,8				AVB			1,7		2,7		420p/5mm³	
56													DIED

* 5 nymphes *R. prolixus*, 5*th* instar, each test.
** 3 tubes Warren medium.
*** Guerreiro & Machado (CFT) — Quantitative (Waldsworth, Maltner & Maltner). A and B dif. labs. Positive > 1,9.
**** 5 mice 10g, each test.
***** Before inf. Strout's technique (19). After inf. Pizzi & Brener technique (4).

(A) 2,9 X 10^6 parasit. *PF*. (B) 3.000 p.Y / gr. body weight.
ABV auricle — ventricular block. T wave inversion (V_1 V_3 V_6).