

NOTES ON THE TREATMENT OF CANINE PIROPLASMOSIS WITH TRYPAN-BLUE.

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PIROPLASMOSIS of the dog is a disease due to the presence of an endoglobular parasite, *Piroplasma canis*, transmitted by a tick, *Haemophysalis leachi*. Thanks to the studies of *Professor Nuttall* and *Dr. Graham-Smith*, it is known how the reproduction of the parasite is effected in the blood of the patient, and also that the simple pyriform shapes (last phase of life-cycle) reaches its final development and leaves the red blood corpuscles in which it develops, in order to immediately get into another not infected one, where it will take a round form. It is owing to these rapid transitions that the parasites are seldom found free in the blood, and infected red blood corpuscles with a single pyriform parasite are rarely present. In severe cases, and towards death, free parasites are frequently noted in the blood. Their presence is due to the rapid destruction of red blood corpuscles.

Forms undergoing division are also rare, because the process is very rapid. Those most frequently found are round or irregular forms and the double pyriform parasites. These are the phases of the cycle in which the development is slower.

Several different forms of the parasite can be observed in a red blood corpuscle at the same time; for instance, three pyriform and one round; two pyriform and one round; four, eight, sixteen, and even twenty-eight pyriform; two or four round; one pyriform and one in division; one or two in division, etc.

It is well known that this disease is very fatal, and natural recoveries are very rare. The medicines tried up to recent times have proved to have but little influence. The different salts of quinine, soamine, methylene blue, naphthylamine blue, tartar emetic, atoxyl, arsenical compositions, etc. were all tried, but without satisfactory results. In 1909, however, *Professor Nuttall* and *Dr. Hadwen* tried a new drug, and they published the results of their treatment in the "Journal of Parasitology". This treatment consists in injecting either subcutaneously or intravenously a watery solution of Trypan-blue—a compound of toluidine and amido-naphthol-sulfonate of sodium. The injection is made in the usual way, and conforming to the rules of asepsis.

The results obtained by the said authors are of a nature which entitle them to state that Trypan-blue as a therapeutic agent for the treatment of canine piroplasmosis is preferable to any other agent known.

The experiments have been continued by several other investigators, including *W. Jowett*, Veterinary Department, Capetown, who, in the *Agricultural Journal of the Cape of Good Hope*, Vol. XXXV, No. 4, 1909, and Vol. XXXVI, No. 5, 1910, published an account of the excellent results obtained by him. This author advises the intravenous injection of the medicine in order to avoid the formation of abscesses, which usually occur in cases of subcutaneous injections, although these abscesses, when cut into in due time and properly disinfected, rapidly heal up.

Whether Trypan-blue is injected intravenously or subcutaneously in a few hours it stains all the tissues a more or less deep bluish colour, also the urine

is stained, and it remains in the body for a few days, then gradually disappears first of all from the mucous membranes of the mouth, and last of all from the skin of the lower part of the abdomen. An autopsy of cases which have died whilst under treatment reveals a bright-bluish colour of all tissues.

During my stay at the Bacteriological Laboratory, Onderstepoort, I was entrusted by Dr. Theiler with the continuation of some experiments which had been started by *Mr. Morpheu*, but on account of his departure he was unable to bring to a final conclusion. For these experiments thirty dogs were injected subcutaneously on the 19th April, 1910, with 2 c.c. of blood of dog 955, suffering from piroplasmosis. Of these, twenty received 5 c.c. of Trypan-blue—a 1 per cent. watery solution—subcutaneously, and the remaining ten were used as controls. The injections of Trypan-blue were made simultaneously in the first lot "A", and in the subsequent lots "B" to "K" at intervals of from one to nine days. One animal, No. 955, was treated with Trypan-blue the day after the parasites appeared in the blood.

The following table gives the results obtained:—

TABLE 1.

INJECTION OF VIRUS ON THE 19TH APRIL, 1910.

Lot.	Number of Dog.	Date of Injection of Trypan-blue.	Date of the First Appearance of the Parasite in the Blood.	Result.	Remarks.
A	941 924	19/4/10 19/4/10	Not noted 29/4/10	Recovered Died of piroplasmosis on the 3/5/10	Anisocytosis present. Piroplasms were never frequent in the blood.
B	917 918	20/4/10 20/4/10	6/5/10 Not noted	Died of piroplasmosis on the 6/5/10 Recovered	Piroplasms not frequent. Anisocytosis present.
C	919 921	21/4/10 21/4/10	Not noted Not noted	Recovered Died from anaemia on the 6/5/10.	Anisocytosis present.
D	922 923	22/4/10 22/4/10	4/5/10 Not noted	Died of piroplasmosis on the 10/5/10 Died of piroplasmosis on the 7/5/10	Piroplasms rare in the blood; very frequent in the spleen. Autopsy revealed typical lesions of piroplasmosis.
E	928 930	23/4/10 23/4/10	Not noted 6/5/10	Recovered Very sick	Anisocytosis present. Piroplasms frequent in the blood.
F	931 932	24/4/10 24/4/10	7/5/10 9/5/10	Sick Died of piroplasmosis on the 14/5/10	Piroplasms rare. Piroplasms frequent and free in the blood.
G	936 937	25/4/10 25/4/10	9/5/10 Not noted	Sick Recovered	Piroplasms very rare. Anisocytosis present.
H	939 943	26/4/10 26/4/10	Not noted 6/5/10	Recovered Sick	Anisocytosis present. Piroplasms rare.
I	948 952	27/4/10 27/4/10	14/5/10 6/5/10	Sick Sick	Piroplasms rare. Piroplasms rare (free in blood on 14/5/10).

Lot.	Number of Dog.	Date of Injection of Trypan-blue.	Date of the First Appearance of the Parasite in the Blood.	Result.	Remarks.
K	954	28/4/10	Not noted	Recovered	Anisocytosis present.
L	955	19/4/10	18/4/10	Died of piroplasmosis on the 14/5/10	This animal served as the origin of the strain of virus and had been injected with virus on the 8/4/10.
Controls.	940	—	26/4/10	Died of piroplasmosis on the 14/5/10	Piroplasms free in the blood.
	947	—	25/4/10	Died of piroplasmosis on the 28/4/10	Autopsy also revealed nephritic lesions.
	949	—	Not noted	Died as a result of an internal injury on the 25/4/10.	
	950	—	27/4/10	Died of piroplasmosis on the 2/5/10	Piroplasms very frequent.
	951	—	26/4/10	Sick	Piroplasms very rare.
	980	—	12/5/10	Died of piroplasmosis on the 13/5/10	Piroplasms very rare in the blood; frequent in the spleen.
	981	—	5/5/10	Died of piroplasmosis on the 13/5/10	Piroplasms rare in the blood and spleen.
	982	—	28/4/10	Died of piroplasmosis on the 29/4/10	Piroplasms rare in the blood; frequent in the spleen.
	983	—	1/5/10	Died of piroplasmosis on the 7/5/10	Piroplasms frequent.
	984	—	20/4/10	Died of piroplasmosis on the 16/5/10	Piroplasms rare.
	985	—	28/4/10	Sick	Piroplasms very rare.

From the examination of this table it can be seen that from twenty-one dogs treated with Trypan-blue only six died from piroplasmosis, that is, 29 per cent., whilst of the ten controls only two survived, a mortality of 80 per cent. Consulting the column headed "Date of the first appearance of the parasite in the blood", it will be noted that in dogs treated with Trypan-blue the parasite appeared generally within fifteen to twenty-five days after injection of the virus, whilst in the controls it was noted as a rule within six to twelve days. Accordingly it can be seen that Trypan-blue in the first instance not only lengthens the incubation time of the disease, but also has preventive effects, as from the dogs injected with the solution nine did not show the parasite at all. Finally, from the columns headed "Results" and "Remarks", it follows that in the injected dogs the course of the disease was of a benign character, excepting the two cases (930) and (952), and ended in recovery. Attention has to be drawn to the fact that of the lots "E" to "K" only a mortality of one in eleven occurred, or 9 per cent. Accordingly the later the Trypan-blue is injected after the virus, but before the parasites have appeared in the blood, the more effect it has on the presence of them; in other words it has more preventive than curative value.

Apparently the Trypan-blue had no effect on dog 955.

On the 19th May, 1910, all the survivors of the first experiment were again injected subcutaneously with 5 c.c. blood of dogs Nos. 985 and 951 in order to test the immunity obtained. The following table shows the origin of the blood and the results obtained:—

TABLE 2.

Lot.	Number of Dog.	Origin of Blood Injected.	Date of the First Appearance of the Parasite in the Blood.	Results.	Remarks.
A	918	Dog 985. Piroplasms present from the 28/4/10 till 14/6/10. Recovered.	6/6/10	Recovered	Piroplasms absent from the 11/6/10.
	919		28/5/10	Died of piroplasmosis on 1/5/10	Piroplasms frequent.
	928		1/6/10	Recovered	Piroplasms absent from the 5/6/10.
	930		6/5/10	Died of piroplasmosis on the 30/5/10	Piroplasms frequent.
	931		9/5/10	Recovered	Piroplasms absent from the 18/6/10.
	936		9/5/10	Died of piroplasmosis on the 25/5/10	Piroplasms frequent five days after injection.
	967 (Control)		30/5/10	Recovered	Had a severe attack, showing many free parasites in the blood.
	966 (Control)		25/5/10	Bled to death ..	Piroplasms very frequent and free in blood; animal was dying when killed.
B	937	Piroplasms present from the 26/4/10 till 17/6/10. Died of Piroplasmosis.	Not noted	Died from enteritis on the 10/6/10.	Autopsy revealed typical lesions of piroplasmosis. Piroplasms absent from the 25/5/10 to the 28/5/10; present from 30/5/10, but always very rare. Piroplasms absent from 25/5/10 to 29/5/10; present from 30/5/10, but never frequent. Piroplasms very frequent on 24/5/10; bled to death on 25/5/10. Piroplasms very frequent and free in blood. Piroplasms very frequent and free in blood.
	939		Not noted	Died of piroplasmosis on the 22/5/10	
	943		6/5/10	Recovered	
	948		14/5/10	Recovered	
	952		6/5/10	Bled to death ..	
	954		Not noted	Recovered.	
	968 (Control)		2/6/10	Died of piroplasmosis on the 20/6/10	
	968 (Control)		27/5/10	Died of piroplasmosis on the 12/6/10	

From the column headed "Results" it can be seen that from the seven dogs which in the first experiment did not show the parasite in the blood (Nos. 918, 919, 928, 937, 939, 952, and 954) only two died of piroplasmosis. Dog 937 died of enteritis twenty-two days after injection, and did not show parasites in the blood. Of those that had previously been suffering from the disease three died, but only 936 can be considered to have died from the second or test injection, as dog 930 showed piroplasms frequently, and dog 952 had shown it free in the blood four days before the second injection.

SUMMARY.

Of the fifteen dogs used as controls in the experiments ten died of piroplasmosis, that is, 66 per cent.

Of the twenty animals which were treated simultaneously with the injection of virus or during the incubation time six died of piroplasmosis, or 30 per cent.

The one dog which was treated with Trypan-blue the day after the parasites appeared in its blood died of piroplasmosis.

Of the thirteen animals which recovered or which resisted the infection and were reinjected a second time with virulent blood, four died. In two instances the dogs did not show any parasites in their blood, and the other two animals showed the parasites as the result of the first injection. As a matter of fact the death of these two animals must still be put down to the first injection, since parasites were never absent in the blood. Accordingly the percentage of deaths from the first injection would increase to 40 per cent.

This high mortality would not speak highly in favour of the therapeutic value of Trypan-blue but for the fact that medicines vary in their influence according to the doses applied, and according to the age, breed, weight, etc., of the animal. In all our experiments the dose was the same for all animals, which were of different ages, breed, and weight, and it must be mentioned that those which recovered were the smaller ones; it must also be mentioned that no extra treatment in the way of additional or nutritious feeding and comforts was given to the sick animals as would be the case, for instance, in a private practice.

Accordingly, taking all these facts into consideration, we cannot come to any conclusion other than that Trypan-blue is the most powerful agent that therapeutics can offer at present for the treatment of canine piroplasmosis, and it will undoubtedly render valuable services in practice.

We are further justified in concluding from these experiments that Trypan-blue possesses certain preventive qualities, and that the animals which recovered under its treatment have acquired immunity for a period which has not yet been determined.