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Transmission of the South African Strain of Dourine to Laboratory Animals.

By D. A. HAIG and (the late) A. S. LUND, Section of Bacteriology, Onderstepoort.

In South Africa dourine is present in a highly insidious form. The disease was tentatively diagnosed in 1914 and this diagnosis was confirmed in 1918 when sera were sent to Watson in Canada for complement fixation tests. Yet it was not until 1935 that the first trypanosome was found in an infected equine (Parkin and van Rensburg, personal communication).

Walker (1918) carried out a large number of transmission experiments with the South African strain. All were negative except in the case of one puppy inoculated intraperitoneally with 300 c.c. blood from an infected mare. In this puppy he found a few trypanosomes 13 days after inoculation. Later de Kock *et al* (1939) found numerous trypanosomes in vaginal washings of newly infected mares, but they were unable to transmit them to white mice.

Numerous workers in other parts of the world have stressed the difficulty of transmitting field strains of dourine to laboratory animals. Watson (1920) after many fruitless attempts did infect the white mouse. From the white mouse other laboratory animals were easily infected; and in the white rat the concentration was so high that a satisfactory antigen for the complement fixation test could be prepared. Ciuca (1933) showed that the rabbit testicle could be infected and he used this as a means of diagnosis, but he could not maintain the strain in serial passage. Domilescu (1938) using Ciuca's method of testicular inoculation, infected rabbits, dogs and rats. He found it advisable to paralyse the reticulo-endothelial system and states that splenectomy did not increase the susceptibility of the rat. His strain was in its third passage in rabbits and its second in dogs and rats.

Parkin of this institution [see his article on Demonstration and Transmission etc. (this Journal)] was able to transmit the disease from equine to equine by the intravenous injection of large amounts of blood. In this way he was able to increase very greatly the number of parasites found in the blood. These equines offered an excellent source of infective material and it was from them that the following transmissions were made.

I. *Transmission to the rabbit testicle.*

Following on the work of Ciuca (1933) we injected 1 c.c donkey plasma containing about 40 trypanosomes into the testicle and scrotal cavity of a rabbit. When examined two weeks later, aspirated fluid from the scrotum was rich in trypanosomes. 0.2 c.c. of this fluid was injected into the scrotal cavity of a fresh rabbit and in this way the strain was passaged 50 times when the experiment was discontinued. Infected testicles showed a slight

swelling a few days after inoculation. This swelling slowly increased in size until about the twentieth day, when the testicles were almost twice their normal size. The swelling then gradually decreased and left the testicles small and hard and the scrotum thick and wrinkled. Sometimes the subsidence of the swelling was accompanied by irritation, and in these cases the animals bit at the scrotum and so caused large sores.

Trypanosomes were frequently found in the fluid of the peritoneal cavity at the height of the testicular reaction, but fresh rabbits could not be infected with this material, either by intraperitoneal or intravenous injection. Rarely were the parasites found in the blood and then only in small numbers. Thirty c.c. of such blood inoculated intraperitoneally or intravenously did not transmit the disease to other rabbits. Attempts to produce an invasion of the blood stream of infected rabbits by intravenous injections of large amounts of India ink or disodium phosphate and calcium chloride as recommended by Domilescu (1938) were unsuccessful, nor did splenectomy or insulin shock reduce their resistance.

II. *Transmission to the Dog.*

A dog inoculated intraperitoneally with minced rabbit testicle showed a few trypanosomes in its blood 9 days later. They could be found as long as 3 months after injection but other dogs sub-inoculated with blood from this dog remained negative. Normal and splenectomised white rats were then inoculated with 3 c.c. of the infected dog's blood. A few trypanosomes were found in the deposit of centrifugalised plasma from the splenectomised rats. The trypanosomes were maintained through two further passages in splenectomised rats and were then lost.

III. *Transmission to White rats.*

From the above experiment it seemed that splenectomised white rats offered some promise as susceptible animals. One splenectomised and two normal rats were inoculated intraperitoneally with 5 c.c. plasma from a donkey showing about 40 to 50 trypanosomes per c.c. This and all subsequent inoculations in splenectomised rats were done one hour after the operation. Thirteen days later the rats were examined and a large number of trypanosomes and bartonellas were found in the blood of the splenectomised one. They were not found in the normal rats. Four further splenectomised rats were then subinoculated from the infected one. Trypanosomes were numerous in their blood four days later. The strain was passaged six times in splenectomised rats, after which normal rats could be infected. These usually showed their greatest concentration of parasites on the fourth day after inoculation after which they rapidly disappeared. Subsequent splenectomy did not bring about relapses. Bartonellosis caused considerable mortality among the splenectomised rats, but did not interfere with the passages for which at least four rats were used each time. After the twentieth generation in normal rats occasional deaths occurred and after a further twenty passages almost all the rats died in four or five days.

The experiment was repeated with blood from another infected donkey. Here in the first passage in splenectomised rats trypanosomes were frequent as early as four days after inoculation.

As in the case of the rabbit testicle strain, no morphological difference could be found between the rat strain and the parent strain. Nor could it be distinguished from our imported antigen strain.

IV. *Transmission of the rat passaged strain to other laboratory animals.*

Of five white mice inoculated intraperitoneally with 1 c.c. heavily infected rat blood, two showed numerous trypanosomes in their blood 3 days later. The parasites were readily transmitted to fresh mice. The strain has now been passaged 50 times in mice and kills in two to three days.

Guinea pigs were refractory. Intraperitoneal inoculation of 10 c.c. infected rat or mouse blood resulted in the appearance of only a few parasites on the day after inoculation. These rapidly disappeared and sub-inoculations were negative. As the number of trypanosomes injected into the guinea pigs was enormous those found were probably only survivors of the original moculum. Splenectomy did not decrease the resistance of the pigs nor did concurrent infections of anthrax or salmonellosis.

Two rabbits inoculated each with 20 c.c. rat blood intravenously remained negative.

V. *Serological Comparison of the South African Rat-Passaged Strain with the imported antigen strain.*

By the fifteenth generation of the trypanosomes in rats their concentra- in the blood was sufficient for the preparation of an antigen for compliment fixation tests. This antigen was prepared, as is our routine antigen, by the method described by Watson (1920). Tests done on sera from ten infected and five normal horses gave identical results when either antigen was used.

Discussion and Summary.

These results show that the South African strain of dourine like the chronic form of the disease in other countries is not readily transmitted to laboratory animals. But where the invasive power of the strain has been boosted by serial blood passage in equines it is easily transmitted to rabbit testicles and to white rats provided the rats are splenectomised.

The rabbit-adapted parasite remains localised and shows little tendency to enter the blood stream. In the splenectomised white rat, the parasites rapidly increase in virulence and are soon adapted to normal white rats. The strain has been passaged over 50 times in normal white rats. Guinea pigs and rabbits, are resistant. The strain differs in this respect from our imported one which is virulent for these animals. What part bartonellosis plays in rendering splenectomised rats susceptible to the disease is not known.

Both the rabbit-testicle and rat-adapted parasites were indistinguishable morphologically from the original strain, and from our imported strain. An antigen prepared from the rat strain gave the same results as our routine antigen when tested against sera from normal and dourine infected horses.

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