The verification of the existence of a negative phase in the prepatent period of canine babesiasis transmitted by ticks

by

W. Lobato Paraense

(With one table)

INTRODUCTION

The present-day knowledge regarding the biology of Babesiidae indicates the existence of several analogies between those parasites and the Plasmodiidae. Both families of sporozoa show some common characteristics closely relating them within the zoological system.

In the classification followed by Wenyon (1926) the hemopiroplasms form a suborder which comprises the two families Haemoproteidae and Plasmodiidae, besides the piroplasmata which include the two families Babesiidae and Theileriidae.

In the system adopted by Kudo (1946) the hemopiroplasms constitute an order formed by the three families Plasmodiidae, Haemoproteidae and Babesiidae. In the latter the genera Babesia and Theileria are included.

In the Haemoproteidae, a schizogony cycle occurs in some tissue cells of the vertebrate. This is followed by a sexual cycle in the blood corpuscles.

In the Plasmodiidae, a schizogony cycle occurs in tissue cells and is followed by a schizogony and a sexual cycle in the red blood cells.

In the Theileriidae, a schizogony cycle occurs in tissue cells, and from the resulting merozoites the ones which penetrate into the red blood cells behave like gametocytes. The biological cycle of a theilerid in the vertebrate host is quite comparable to that of a hemoproteid which does not produce pigment during its blood phase, as happens to the genus Leucocytozoon. In both families, Theileriidae and Haemoproteidae, the infection is not transmitted by blood inoculation due to the absence of multiplicative forms of the parasite in the blood cells.
On the other hand, in the Babesiidae, as in the Plasmodiidae, the inoculation of blood does transmit the infection due to the existence of multiplying parasites in the red cells. In fact, up to the present, no one has been successful in distinguishing, amongst the parasites which occur in babesiasis, those forms that might correspond to the gametocytes of the plasmodia, although some authors admit their existence.

In order to orientate our investigations on the Babesiidae, we admitted as a working hypothesis that on equivalence might exist between those parasites and the Plasmodiidae. Just as the Theileriidae might be considered as equivalent to unpigmented Haemoproteidae, so the Babesiidae might be roughly compared to plasmodia that were unable to produce pigment.

The present work was undertaken with the object in view of verifying the existence of a negative phase in the blood during the initial part of the prepatent period of the babesiasis transmitted by ticks. Such a negative phase is now proved to exist in the malarial infections induced by sporozoites.

MATERIAL AND METHODS

PARASITE STRAIN

The strain used in the present experiments was isolated from a stray dog, in Rio de Janeiro, in May 1948. The animal was given the number 92 in a survey on the occurrence of dog babesiasis in this city (Paraense and Vianna, 1948). The strain is maintained in the tick Rhipicephalus sanguineus and in dogs.

TICK INFECTION

In January 1949 a dog, 37 days old, was inoculated intravenously with blood from another dog infected with our strain of B. canis. On the same day 100 adult R. sanguineus were put on the recently inoculated dog, which was kept in the tick culture room at a temperature of 24-25 C. degrees and high relative humidity (above 95 per cent.). On the 3rd day after inoculation the parasites were first detected in the red blood cells, and thereafter an intense parasitemia developed with ensuing death on the 17th day after inoculation. Within 7 to 10 days after inoculation 21 engorged females fell from the dog. The larvae issuing from their eggs fed on normal dog which remained uninfected. The nymphes similarly fed on a normal dog which showed blood parasites on the 6th day after being infested with the ticks.
More than 1800 imagines were finally obtained from which 1600 were used in the present experiments.

**EXPERIMENTAL ANIMALS**

Twelve dogs were used, two of them being infested with ticks and the other 10 inoculated intravenously with blood from the former two.

All the dogs were born in the laboratory, of bitches previously disinfected from ectoparasites, and were kept in large metallic net floored cages, the latter resting on large metallic trays containing water. This device keeps the animals free from undesired wingless arthropods.

In order to avoid the dogs eating the ticks fixed on them, the two animals infested with ticks were kept muzzled during the whole period of infestation. The muzzle was removed twice daily, at each meal, and the animals were watched while feeding in order not to allow them to eat the ticks.

**BLOOD SMEARS**

Common blood smears from each dog were made daily at 3 p.m. throughout every experiment. The smears were stained with Giemsa's stain and examined for parasites in places where the blood cells were regularly distributed, showing some 200 corpuscles per field (Zeiss binoc. 1.5, oc. 5, obj. HI 100).

**EXPERIMENTS AND RESULTS**

Dog no. 1, six months old, was infested with 1500 imagines.

Five dogs, nos. 1-A, 1-B, 1-C, 1-D, and 1-E, were inoculated intravenously with 5 ml of blood of dog no. 1, respectively 24, 48, 72, 96, and 120 hours after the latter had been infested with ticks. The animals inoculated with blood belonged to a litter which was 49 days old when the first inoculation was made.

Blood parasites were first observed in dog no. 1 in the smear made on the 6th day after infestation with ticks.

Dogs nos. 1-A, 1-B and 1-C remained negative during 30 days of observation.

These negative results indicate the absence of parasites in the blood of dog no. 1 until the third day after its infestation with ticks.

Dog no. 1-D, inoculated on the 4th day, showed blood parasites on the 4th day after inoculation, dying from intense parasitemia on the 7th day.
Dog no. 1-E, inoculated on the 5th day, became positive on the 3rd day after inoculation, dying from acute infection on the 8th day.

Dog no. 2, five months old, was infested with 100 imagines.

The conditions of this experiment were identical to those of the previous one, there being only two exceptions: the number of ticks that were put on the dog no. 2, and the age of the animals inoculated with blood being 45 days at the beginning of this series of inoculations.

The dogs inoculated with blood took the nos. 2-A, 2-B, 2-C, 2-D, and 2-E.

Dog no. 2 was positive on the 6th day after being infested.

Dogs nos. 2-A, 2-B, and 2-C were negative until the 30th day after blood inoculation, as was the case with the corresponding animals of the previous experiment.

Dog no. 2-D became positive on the 4th day after inoculation, dying on the 10th day.

Dog no. 2-E became positive on the 3rd day and died on the 9th day.

The results of both experiments were then exactly the same as far as blood infectivity of the donor animals was concerned (see table 1).

**Table 1**

Tests made during the prepatent period, by inoculation of blood from dogs nos. 1 and 2, infected with *R. canis* from ticks, into normal puppies.

<table>
<thead>
<tr>
<th>DONOR DOG N.</th>
<th>1st DAY OF INOCULATION (AFTER DONOR'S INFESTATION)</th>
<th>PARASITIZED ERYTHROCYTES PER 100 FIELDS</th>
<th>DAYS AFTER INOCULATION</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1-A</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-B</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-C</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-D</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-E</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-A</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-B</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-C</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-D</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-E</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* Infected with 1500 imagines. Parasitized erythrocytes first observed on the 6th day.

*b* Infected with 100 imagines. Parasitized erythrocytes first observed on the 6th day.
DISCUSSION

The experiments just described indicate that in Babesia the sporozoites injected by the tick do not enter the red blood cells; therefore, by analogy to the other hemoparasidae, the natural conclusion is that they do penetrate into tissue cells of the vertebrate.

Up to the 3rd day after sporozoite inoculation the babesia accomplishes a preerythrocytic development in the host tissues, at the end of which the erythrocytic forms are produced. In B. canis the latter forms escape into the blood stream on the 4th day of the prepatent period. During the remaining days of the prepatent period the erythrocytic forms multiply so rapidly as to be easily found by microscopical examination of the blood on the 6th day.

We find this interpretation to be quite acceptable because it harmonizes with the facts occurring in all other families of hemoparasidae.

In his paper on the development of B. canis in the tick, Regendanz (1932) gives another interpretation. It is well known that in tick-borne babesiasis the appearance of the parasites in the blood circulation only occurs after the 6th day. On the other hand, in those cases inoculated with infected blood, the parasites are already found about the 3rd day. Furthermore, the multiplication of the babesia in the salivary glands of the tick is only begun when it begins to suck the blood. Considering these facts, Regendanz suggests that the ticks only begin to inoculate the sporozoites in the vertebrate on the 3rd or 4th day after they begin to suck, and that the sporozoites invade the red blood corpuscles, where multiplication takes place; on the 6th day after the beginning of the bite (3rd after blood invasion) the parasitemia becomes patent.

Thus, according to Regendanz, the prepatent period after sporozoite inoculation may be divided into: 1. a negative phase of the blood, without any parasite in the vertebrate body, with a duration of 3 to 4 days (*); 2. a subpatent phase, during which the parasites multiply within the red cells but, however, are not yet detected by microscopical examination, with a duration of about 3 days.

According to our interpretation, the prepatent period should be divided into: 1. a negative phase of the blood, with parasites multiplying in tissue cells, having a duration of at least 3 days; 2. a subpatent phase, with parasites multiplying in the red corpuscles but not yet detected by microscopical examination, having a duration of about 3 days.

(*) Regendanz admitted such a negative phase by illation, and not as the result of experiments by blood inoculation during the prepatent period.
Notwithstanding the interpretation that we have given to the negative phase demonstrated in the present paper, we are preparing suitable material for further experiments that will permit us to verify if ticks are unable to inoculate sporozoites during the earlier days after attaching themselves to the vertebrate host. In this order of ideas it should be remembered that the infection was transmitted by the nymphs of the same batch used for the experiments with imagines. We understand that the imagines emerged from infective nymphs must have sporozoites in their salivary ducts ready to be inoculated at the exact moment when the tick starts to feed.

SUMMARY

Two dogs were infected with Babesia canis by the bite of 1500 and 100 adult Rhipicephalus sanguineus respectively. Both dogs showed blood parasites after a prepatent period of 6 days. Through the prepatent period 5 ml of blood were taken daily from each dog and inoculated intravenously into 10 puppies. From the latter, those which were inoculated on the 1st, 2nd and 3rd day of the prepatent period remained negative, while those inoculated on the 4th and 5th days became infected with Babesia.

In accordance with the knowledge obtained regarding the preerythrocytic development in the other hemosporidias, the results of our experiments may be interpreted as indicating the existence of an early developmental phase of the babesia in tissue cells of the vertebrate host, corresponding to a negative phase of the blood, and of at least 3 days duration.

REFERENCES

Kudo, R. R.

Paraense, W. L. & Y. L. Vianna

Regendanz, P.

Wenyon, C. M.