

SUSCEPTIBILITY OF BATS TO INFECTION WITH THE
HORSE TRYPANOSOME, *TRYPANOSOMA HIPPICUM*
DARLING, IN PANAMA

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Some investigations on the potential relation of bats to various human and animal diseases have recently been undertaken. During this work a few experiments were carried out to determine the susceptibility of these flying mammals to infection with *Trypanosoma hippicum*, the causative agent of the "murrina" of horses in Panama.

Several species of the more common bats that are available in Panama were collected and used in these studies. The trypanosomal infected blood was administered to the bats by three methods, being injected intraperitoneally, fed by mouth and passed directly into the stomach through a rubber tube.

INFECTED BLOOD INJECTED INTRAPERITONEALLY

Experiment 1. On January 14, 1931, two of the large spear-nosed bats, *Phyllostomus hastatus panamensis* Allen, were captured in the Chilibrillo caves and brought to the laboratory. Their blood was examined daily for a period of seven days with negative results. The thick-drop-blood-film method was used in these examinations and in all subsequent ones made in connection with the bat experiments reported in this paper. On January 21, each of the two bats was inoculated intraperitoneally with 0.5 cc. of blood, taken from the heart of Guinea Pig 28 in which *Trypanosoma hippicum* were very numerous at this time. These inoculations were made at 3 p.m. Both bats became infected and it was rather surprising to find trypanosomes in their blood within 18 hours after the inoculation. A blood film taken from Bat H-2 at 9 a.m. on January 22 showed 14 trypanosomes. The infection in this bat increased until by January 27

more than 600 trypanosomes were present in a microscopic field. This bat died during the night of the 28th. A blood film taken from the second bat, H-4, at 9 a.m. on January 22 revealed 15 trypanosomes. On the following day this number had increased to 90, and on January 24 more than 400 were counted in a film. On January 31 a blood film showed more than 600 trypanosomes. This bat died February 1, the eleventh day after inoculation.

Experiment 2. Three short-tailed bats, *Hemiderma perspicillatum aztecum* (Saussure), were used in this experiment. After the blood of these bats had been examined on seven consecutive days with negative results, each of them was inoculated intraperitoneally with 0.5 cc. of heart's blood from infected Guinea Pig 28 at 3 p.m. on January 21, which was the same time that the bats used in Experiment 1 were inoculated. Two of the short-tailed bats showed trypanosomes in their peripheral blood 18 hours after inoculation, one of them having more than 400 trypanosomes to a film at this examination. The blood of the third bat did not reveal trypanosomes until approximately 42 hours after inoculation. One of the bats died on the second day after inoculation and one on the third day, while the remaining one survived for a period of seven days. The blood of this last was showing numerous trypanosomes in each microscopic field in a film taken shortly before death.

Experiment 3. Three small yellow bats, *Rhogeessa tumida* Allen, were used on this occasion. These bats are about the smallest in size of any found in Panama and are believed to be strictly insectivorous. Seven of them were captured in the attic of a house at Balboa, in the Canal Zone, and sent to this laboratory. Four of them had been injured during capture, but the other three appeared to be in good condition and were used in this experiment. Each was inoculated intraperitoneally with a drop of blood taken from the ear of infected Guinea Pig 113. A blood film taken from the guinea pig at this time showed an average of about 150 trypanosomes to a microscopic field. The drop of blood received by each bat was diluted with saline solution before being injected. These inoculations were made at 11 a.m. on June 24. Blood films taken at 4 p.m. (5 hours later) showed trypanosomes present in the peripheral blood of each bat. One showed 5 trypanosomes in a film at this first examination, and 10 in a

film taken at 9 a.m. the next day. The second bat showed 9 trypanosomes in a film taken 5 hours after inoculation and 147 in a film taken at the end of 22 hours. The third bat presented only one trypanosome in a film taken at the end of 5 hours and died before the morning of June 25. The first two bats died during the night of June 25. The death of these three bats within 48 hours after inoculation may have been due as much to shock and injury received while they were being inoculated or while blood was being taken from them, as to the effects of the trypanosomes. No examination had been made of the blood of these bats previous to their inoculation, as they were so small and delicate that it was expected that they would survive only a short time in captivity. The trypanosomes found in the blood of the bats after the inoculation were so typically *Trypanosoma hippicum* that there was but little chance of any trypanosome that might have been present from a natural infection being mistaken for this species.

Experiment 4. Four long-tongued bats, *Glossophaga soricina leachii* (Gray), were examined for blood parasites on June 26, with negative results. At 10 a.m. on June 27 they were each inoculated with 0.8 cc. of blood taken from the heart of experimentally infected Bat 28. Blood films taken from this infected bat at this time showed more than 50 *Trypanosoma hippicum* in each microscopic field. Three of the four bats were dead by the following morning, probably due to injury or shock received during inoculation. A blood film taken from the fourth bat at 10 a.m. on June 28 showed more than 100 trypanosomes. This number increased to more than 200 per film on June 29, and to 300 on June 30. This bat died during the afternoon of June 30.

Experiment 5. Two of the spear-nosed bats, *Phyllostomus hastatus panamensis* Allen, whose blood had been examined daily for five days with negative results, were each inoculated with 0.3 cc. of blood taken from the ear of infected Monkey 318 at 3:30 p.m. on August 8. The blood of one of these bats was positive at 10 a.m. the next day, when 8 trypanosomes were counted in a film. The infection in this bat increased in intensity until many trypanosomes were present in each microscopic field in the blood films taken each day. This bat survived until September 4, a period of 26 days after inoculation. The second bat did not reveal trypanosomes in its blood until August 11,

three days after inoculation. The infection in this animal gradually became more intense until the daily blood films showed many trypanosomes in each microscopic field. This bat died during the night of August 18.

Experiment 6. Two of the striped-face bats, *Artibeus jamaicensis jamaicensis* Leach, were used for this experiment. Daily blood examinations of these bats for six days gave negative results. They were then each inoculated with 0.3 cc. of blood taken from the heart of experimentally infected Bat 61. A few trypanosomes were present in blood films taken from the two bats 18 hours after the inoculation. Two days later the blood films taken from them contained many trypanosomes in each microscopic field. They both died during the night of the fourth day after inoculation.

Experiment 7. Four of the spear-nosed bats, *Phyllostomus hastatus panamensis* Allen, after having their blood examined daily for six days with negative results, were each inoculated intraperitoneally with heart's blood from experimentally infected Bat 67. Blood films taken from this host bat at the time contained many *Trypanosoma hippicum* in each microscopic field. All four of the experimental bats revealed trypanosomes in blood films taken 18 hours after the inoculation. One of the bats ran a fairly heavy infection and died on the sixth day after inoculation. The other three developed very heavy infections with numerous trypanosomes to be found in each microscopic field, and died on the thirteenth, fifteenth and twenty-sixth days, respectively after being inoculated.

INFECTED BLOOD PASSED DIRECTLY INTO STOMACH THROUGH A RUBBER TUBE

This method was employed with a view to determining if the bats would become infected through the mucous membranes.

Experiment 1. A large *Phyllostomus hastatus panamensis* was used for this experiment. This bat was first immobilized by bandaging it to a narrow strip of wood. A wooden tongue depressor, cut down to about two-thirds of its regular width and having a small round hole bored through the center, was placed in the bat's mouth to hold it open. A rubber catheter of very small caliber was pushed through the hole in the tongue depressor and down in the throat of the bat until

it was believed that the end had reached the stomach. About 0.5 cc. of blood from the heart of infected Guinea Pig 107 was drawn into a Luer syringe. The needle was then removed from the syringe and the glass tip of the latter inserted into the catheter and the infected blood then forced into the stomach of the bat. Films made of the infected blood at this time showed many trypanosomes present. The blood of the bat became positive 48 hours after inoculation. The infection gradually increased in intensity until the death of the bat on the fifth day after inoculation.

INFECTED BLOOD FED BY MOUTH

As there was a possibility of the rubber catheter causing slight abrasions of the mucous membranes while it was being pushed down the bat's throat in the foregoing experiment, it was decided to attempt to inoculate bats by simply feeding them with infected blood. In these experiments the blood was drawn from the heart of the host animal and the needle then removed from the syringe and the glass tip of the latter placed in the mouth of the bat to be inoculated. Gentle pressure on the plunger of the syringe then slowly forced out the blood, which the bat readily swallowed.

Experiment 1. Four bats, *Hemiderma perspicillatum aztecum*, whose blood had been examined with negative results, were each fed approximately 0.3 cc. of blood from infected Guinea Pig 111 on June 13. One of these bats died on June 13 and another died on June 19, neither of them giving any positive blood findings before they died. The third bat revealed trypanosomes in its peripheral blood on June 21 and died the following day. The fourth became positive on June 20 and developed a very heavy infection before it died on June 27.

Experiment 2. Two *Artibeus jamaicensis jamaicensis* were each fed 0.3 cc. of blood from infected Guinea Pig 111 on June 13. One became positive on June 18 and died on June 27. The other became positive on June 20 and died five days later. Both of these bats developed very heavy infections with a great many trypanosomes present in their peripheral blood.

Experiment 3. Two *Phyllostomus hastatus panamensis* were used in this experiment. Each was fed 0.5 cc. of blood from the heart of experimentally infected Bat 61 on August 11. Both became positive

on August 19 and developed heavy infections with numerous trypanosomes present in their blood. One died on August 25 and the other on the 28th.

SUMMARY AND DISCUSSION

1. A number of experiments have demonstrated that bats are readily susceptible to infection with the horse trypanosome, *Trypanosoma hippicum* Darling, in Panama.

2. The infection was produced in the bats by infected blood being injected intraperitoneally; by passing it through a rubber tube into the stomach; and by feeding it by mouth.

3. Five species of bats were used in the experiments and positive results were obtained with each species.

4. The incubation periods in the bats that were inoculated intraperitoneally varied from 5 to 72 hours. More than 68 per cent of the bats became positive as early as 18 hours after inoculation. The bats that were tube-fed with infected blood became positive in 48 hours. The incubation periods in those fed with infected blood varied from five to nine days.

5. Two of the bats survived for twenty-six days after being inoculated, this being the maximum period of longevity of the infected bats. Several died two or three days after becoming positive.

6. The short, fatal course of this equine form of trypanosomiasis in fruit-eating and insect-eating bats indicates that they cannot become very important carriers or reservoirs of the disease in a community. The fact that this trypanosomal infection was successfully passed through the normal mucosa of the alimentary tract in the mouth-feeding experiments makes it highly desirable to apply this method of investigation to the blood-sucking bats that attack horses and mules. We have not yet been able to catch this species of bat in the act of feeding on animals nor have we been able to find their roosting places.¹

¹ Since this article went to press, we have found the roosting place of certain blood-sucking bats, and experiments are now being carried out along the lines indicated.