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EXPERIMENTS IN THE TRANSMISSION OF TRYPANOSOMA HIPPICUM DARLING WITH THE VAMPIRE BAT, DESMODUS ROTUNDUS MURINUS WAGNER, AS A VECTOR IN PANAMA*

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Equine trypanosomiasis (murrina) of Panama is a disease, affecting horses and mules, that has made great inroads upon the equine population of the Isthmus during the past. The causal organism, *Trypanosoma hippicum* Darling, was first discovered in Panama by Darling in 1909; studies concerning it have since been carried on by various investigators at frequent intervals. The mode of transmission has always been one of the most important problems under consideration in these studies. Attempts have been made to find an insect intermediate host of this disease, but to date these efforts have given only negative results.

In 1912 Darling[1] succeeded in infecting a mule by allowing eighteen house flies, *Musca domestica* L., to feed on, and walk about in, fresh blood from a heavily infected guinea pig, and then immediately transferring these flies to a freshly shaved and scarified area on the skin of the mule. While this transmission may be termed a purely mechanical one, it was the only time that positive results have been

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[1] Darling, S. T.: Trans. 15th Internat. Congress on Hygiene & Demography, Sec. VIII (Military, Naval & Tropical Hygiene), p. 10. Washington, 1912.

obtained by using insects in any manner in experiments on the transmission of this disease.

During the past three years a number of horses and mules in the Canal Zone have developed "murrina." The majority of these animals apparently acquired their infection while in pasture, and it was noticeable that many of them showed evidence of having been bitten by vampire bats. The presence of these numerous bites raised a question regarding the possibility of bats acting as transmitting agents, and investigations along these lines were considered.

While seeking for vampire bats, experiments were undertaken to determine the susceptibility of several species of the more common frugivorous and insectivorous bats to infection with *Trypanosoma hippicum*. These studies demonstrated that the bats were readily susceptible to this form of trypanosomiasis and that they died from the infection. It was also shown that they could acquire the infection through the trypanosomes invading the normal mucosa of the alimentary tract when the bats were fed with infected blood. A detailed report of these investigations has been given in an earlier paper.[2]

That Darling considered bats in connection with this disease in 1909 is shown by his statement:[3] "Before the isolation corral was screened, bats visited it and bit one of the infected horses on several occasions on both sides of the neck. Curiously enough, this was the only animal out of three or four in the stable that was bitten. While bats under such circumstances might open a channel for infection by breaking the skin of an infected animal and permitting flies to become infected, it did not seem that in this case, at any rate, they transmitted the infection; but if they had visited non-immune horses and bitten them immediately after visiting and biting an infected horse, the bats would have then possibly become a transferring medium." Unfortunately it was not known or suspected at the time of Darling's investigations that the bats might themselves acquire the disease and play a more important part than causing a mechanical transmission by attacking a clean animal immediately after having bitten an infected one or by causing a break in the skin from which flies might possibly become infected.

[2] Dunn, L. H.: Jour. Prev. Med., 1932, 6: 155–160 (No. 3, May).

[3] Darling, S. T.: Proc. Canal Zone Med. Assoc., 1911, vol. 3, part 2, p. 14.

Although it was shown by our experiments that the frugivorous and insectivorous bats are susceptible to infection with *T. hippicum*, their feeding habits would seem to exclude them from playing any very important part in the transmission of this organism other than through the instrumentality of some invertebrate agent. The vampires, however, must be considered from quite a different view point. They seem to subsist wholly on blood and are apparently indiscriminate in their selection of hosts. They prey on the animal life in the vicinity of their habitations for their sustenance; since domestic animals are quite often the most available in many localities, numerous equines and bovines are nightly attacked by these sanguivorous mammals in order to obtain the blood necessary for their survival.

One might infer, from the number of domestic animals in Panama attacked by vampire bats, that vampires are so numerous in Panama that they could be captured in large numbers. It was only after considerable searching, however, that living specimens of *Desmodus rotundus murinus* Wagner, the species of vampire that appears to be the most common throughout tropical America, were secured. These bats were found in a cave on Taboga Island in the Bay of Panama and also in the Chilibrillo caves about 15 miles by air line from Panama City, in the basin of the Chagres river. After their habitats were located and specimens collected, a series of experiments to determine the possibility of their acting as vectors of "murrina" was carried out in the early part of 1932.

Experiment 1. On January 28, six vampire bats were each allowed to feed on the margin of the ear of Horse 200, which was infected with *Trypanosoma hippicum*. Blood films taken from the horse at this time showed rather a scant infection (only one trypanosome to a slide). Five of the bats fed well and became partly filled; the sixth bit the ear quite viciously several times and lapped up some blood, but did not take enough to cause any noticeable distension. After the bats had fed, they remained at large in the screened stall with the horse. The latter died on February 8. Horse 241, a clean animal, was then placed in the stall. Three of the bats were accidentally drowned in a pail of water in the stall on January 31. The blood of the three remaining bats was examined on February 10 with negative results. A fourth bat was found dead in the pail of water on the morning of

February 11. Blood films taken from the remaining two bats on February 17 were negative. On February 20 the clean horse was removed from the stall and was replaced by infected Horse 227, which at this time was showing an average of about 14 trypanosomes to a thick drop film. This horse seemed to have been bitten on the side of the neck by at least one of the bats on the night of February 20, and between that date and March 5 it showed marks of three bites just below its left fore fetlock; it is quite probable that the two bats fed daily, or nightly, on the horse. On February 29 the blood of the two bats was examined with negative results; on March 4, however, both bats were found to be positive, one showing trypanosomes in each microscopic field and the other having not more than two to a film. On March 5 the infected horse was taken from the stall and the clean Horse 241 put in again. The bat having the heaviest infection was found dead on March 10; it was badly crushed and had evidently been killed by the teeth or hoofs of the horse. On March 14 Horse 241 was removed from the stall and a second clean animal (Horse 222) was put in. The last bat died on March 28, twenty-four days after it was known to be positive. Since neither of the clean horses that were exposed to the bites of these bats ever became positive, this experiment must be considered negative.

Experiment 2. Vampire Bat 8 was allowed to take blood from infected Guinea Pig 244 on three successive days, February 1, 2 and 3, when thick drop films of blood taken from the guinea pig on each day showed numerous *Trypanosoma hippicum* in each microscopic field. The bat was then fed alternately on two clean guinea pigs for four days. Its blood was first found to be positive on February 8, a thick drop blood film showing 20 trypanosomes on that date. It is probable, from the number of trypanosomes present at this time, that the organisms had appeared in the peripheral blood one or two days earlier.

The bat was fed on a clean guinea pig each of the 17 days from February 8, when it was known to be positive, until February 25. The guinea pig bitten by the bat on February 8 died the following day; the guinea pigs bitten on the next three days remained negative. The first animal to become positive was the one bitten on February 12, when an examination of the bat's blood showed an average of about

50 trypanosomes in each microscopic field. Each guinea pig bitten by the bat during the next 12 days became infected, thus giving positive results in 13 animals in succession.

On February 25 the bat was allowed to feed on Horse 256. It fed for 80 minutes and became thoroughly gorged. The lesion made by the bite was very small. A clean guinea pig, used on February 26 for feeding the bat, later became positive. On February 27 the bat took a second meal of blood from the same horse, feeding for 115 minutes and becoming very full. The horse began to show trypanosomes in its blood on March 5, eight days after the first bite of the bat and six days after the second feeding, giving an incubation period of from six to eight days. Of the six clean guinea pigs that the bat fed on between February 27 and March 5, when it died, one became positive.

In all, the bat, after it had become positive, fed on 24 guinea pigs. One of these died so soon after being bitten that it cannot be considered. Of the other 23, fifteen became positive, the incubation period varying from six to twelve days. The length of time that the bat spent in feeding upon each guinea pig that became positive ranged from 40 to 150 minutes; in twelve the feeding lasted an hour or more. The bat lived 26 days after it became infected.

Experiment 3. On March 29, Vampire Bat 18 was allowed to feed on infected Guinea Pig 301. It fed for about two hours and became quite distended. It was later fed on clean guinea pigs and its blood examined daily until it became positive on April 6—eight days after the infective feed. Two films of the bat's blood taken on this date showed five trypanosomes in one and seven in the other. Between April 6 and April 27 the bat was fed either every day or every second day on a clean guinea pig, 14 animals being used, but none of them becoming positive. It was believed that the negative results were caused by the manner in which the bat was feeding.

Vampire bats in captivity usually bite all the later animals on which they feed, in the same area as they bite the first one. In the present experiments each guinea pig for a bat to feed upon was immobilized by being bandaged to a stick, lying on its back with forelegs upward, while the hind legs were bandaged down so that they extended to the rear in a line with the body. The bat was usually held while it was

starting to feed upon a guinea pig for the first time, and allowed to bite only on the dorsal surface of the pig's hind foot. The bat would invariably thereafter feed at this same area of the succeeding guinea pigs and make no attempt to bite them about the head.

Bat 18, of the present experiment, maneuvered during its first bite, however, so that it started to feed on the sole, instead of the dorsal surface, of the hind foot of the first guinea pig, and each of the guinea pigs on which it fed later was bitten at the same place. As each animal was placed in the cage with the bat the latter crept up beneath the left hind foot and made a quick bite, sharp enough to remove a good sized piece of skin near the heel. The blood usually oozed out quite freely, and the bat apparently fed by simply touching its tongue to each drop of blood as it formed at the lower part of the wound. The lightest touch of the tongue seemed to draw the blood into the mouth of the bat. The raw surface of the wound was never licked by the tongue of the bat as would frequently have occurred had the bite been made in another location.

From April 27 to April 30 the bat was fed for the second time on four of the guinea pigs on which it had fed with negative results previously. At the beginning of each of these four feedings, however, the bat was held in such a position as to force it to make the lesion on the dorsal surface of the guinea pig's foot instead of on the sole. Three of these four guinea pigs subsequently became positive. The bat died on May 2, twenty-six days after becoming infected.

Experiment 4. Two young adult, male Vampire Bats 25 and 26, during a period of nine days (April 17–25), were each daily placed in a wire test-tube basket and the latter inverted on the back of infected Horse 256 and held in place while the bats were given an opportunity to feed. During the first four days *T. hippicum* were sufficiently numerous in the blood of the horse to be found in each microscopic field in a thick drop blood film; the next two days none were to be found in the blood films, but they appeared again in scant numbers during the last three days. Bat 26 fed well upon the infected horse each of the nine days. Bat 25 did not take blood nor bite the horse on the first day, but fed daily during the following eight days. The length of time required for the bats to become gorged with blood varied from 20 to 30 minutes.

Bat 26 showed trypanosomes in its blood on April 23, six days after feeding for the first time on the infected animal. Bat 25 became positive seven days after its first infected feed. Since some of the bats used in previous experiments had become positive six days after feeding only once on an infected animal, it would seem that the repeated infected meals in the present experiment did not tend to shorten the incubation period.

The bats were next fed daily for five days (April 26 to 30) on clean Horse 202. During this time the infection in Bat 26 became very acute and by April 30 numerous trypanosomes were present in each microscopic field of its blood films. The infection in Bat 25 developed more slowly and the trypanosomes in its blood did not become numerous enough to appear in each microscopic field until April 30. They continued to increase slowly day by day, however, until they became very abundant. On May 2 both the bats were fed on another clean horse. Bat 26 died during the morning of May 4, eleven days after becoming infected.

During the ensuing fourteen days Bat 25 was fed on eight more clean animals, six horses and two mules, each animal being bitten but once. Either one or two days elapsed between the feedings. The length of time required for the bat to become satiated with blood from these animals at each feeding ranged from 43 to 120 minutes. When fed only on every second day, the bat usually took more blood and became more greatly distended, thus prolonging the time of feeding. Bat 25 died on May 17, twenty-two days after acquiring the infection.

The total number of clean animals used in this experiment was ten. One of these was bitten five times by each bat; another was bitten once by each bat; eight were bitten once by Bat 25. Two of the animals, a horse and a mule, became positive, trypanosomes being first found in the blood, in both instances, ten days after the biting. Bat 25 had fed on the horse for 63 minutes and on the mule for 43 minutes.

Experiment 5. On April 26, Vampire Bat 21 fed on infected Guinea Pig 328 for a brief time, the bat becoming only partly gorged. Blood films taken from the guinea pig at this time showed an average of about 15 trypanosomes in each microscopic field. During the next nine days the bat was fed daily on cold, clean, guinea pig blood

without being allowed to bite an animal. The bat's blood, examined daily, was found to be positive on May 2—an incubation period of six days.

From May 6 to 26, inclusive, the bat was fed on 18 clean guinea pigs, being allowed to take blood from a new animal each day, except on two occasions when two days elapsed between feedings. Unfortunately, four of the guinea pigs died within five days after they were used for feeding. Of the 14 that survived, three became positive, trypanosomes appearing in their blood after incubation periods of 10, 11 and 13 days. The bat died on May 29, nearly 27 days after becoming infected.

Experiment 6. An adult, male, Vampire Bat 28 had been in captivity for a number of days and was being used in connection with some observations on the dietary habits of vampires. On April 25, when a horse infected with *T. hippicum* was being sacrificed, about four liters of its blood were collected and brought to the laboratory. Since this blood was not defibrinated, it soon coagulated. Approximately 45 minutes after it was obtained, a small slice of the clot and about 10 cc. of the serum were placed in the cage with Bat 28. During the next hour the bat drank part of the serum and ate some of the clot. Throughout the following ten days the blood was kept in a refrigerator and a portion of the clot and serum given to the bat each day. On May 3 the bat became positive, with many trypanosomes in its blood. It is quite probable that the first meal of blood was the one causing the infection. This would indicate that the trypanosomes had survived more than 45 minutes of air temperature and left the blood still infective when eaten by the bat. Daily (on May 7, 9, 10 and 11) the bat was fed on a clean guinea pig for periods varying from 43 to 73 minutes. The four pigs all became positive, two after seven days and two after nine days. The bat died May 12, nine days after developing the infection.

DISCUSSION

The present experiments seem to show that a considerable difference exists among individual vampire bats in ability to transmit trypanosomiasis. One bat may transmit the disease much more readily and infect a larger percentage of the animals on which it feeds than does

another, although both bats may be equally heavily infected. The reason for this difference will be sought in further investigations. It is quite probable that the size of the lesion made by the bite of the vampire and the freedom with which the lesion bleeds may be of considerable significance, as seemingly evidenced by Experiment 3. The vampire does not suck the blood, as popularly believed, but takes it up with its tongue, seldom placing its mouth on the wound except when the latter is first made or when the bleeding is very slow. If the wound bleeds freely, the bat simply laps up the blood, hardly touching the tissues, while if the bleeding is scant the bat licks the wound. It is planned to repeat Experiment 1, which gave negative results, with this factor in mind.

I believe this to be the first time that a biological transfer of "murrina" has been accomplished and a true vector identified. The conviction that this sanguivorous mammal, the vampire bat, is a vector of the disease may be of considerable importance, not only in Panama, but also in South American countries where equine trypanosomiasis of a very closely allied nature, if not identical with "murrina," is prevalent. The distribution of trypanosomiasis throughout South America is practically analogous to that of the vampire bat, at least to the extent of the bat being present in the various countries from which the disease has been reported. Since we have not yet been able definitely to evaluate the importance of the vampire as a vector under natural conditions, the foregoing statements are not meant as a criterion on the frequency with which such infections may be caused by this agent. One has only to know, however, that the vampire is readily capable of transmitting the disease, and then to view the many bites present on the equines in pasture in many parts of the country, to realize the possibilities of the bat as an important disseminator. It now seems strange that the vampire has never before been seriously considered in connection with the transmission of "murrina," since it would appear to be the most logical agent to be investigated when compared with invertebrates.

As opportunities permit it is proposed to conduct investigations to ascertain the possibilities of the vampire bat's being a factor in the transmission of other diseases of man and animals in the tropics.

SUMMARY AND CONCLUSIONS

1. The positive results in five out of the six experiments in the transmission of "murrina" (the equine disease produced by *Trypanosoma hippicum* Darling) through the agency of the vampire bat, *Desmodus rotundus murinus* Wagner, definitely prove the vampire bat to be a vector of the disease.

2. The eight bats used readily acquired the disease, either by taking a meal of blood directly from an infected animal or by consuming blood that had previously been taken from an infected animal.

3. The incubation periods in the bats were from six to eight days.

4. The disease proved fatal to all the bats.

5. The interval between infection and death in the bats varied from nine to 27 days. Since the bats seem to feed every night, this long interval gives ample opportunity for them to bite many animals after they—the bats—have acquired the infection. The bats' appetite seems to be in no way impaired by the infection, but, on the contrary, increased, as is the case with equines that have the disease.

6. In the five experiments with positive results, the following clean animals became infected through the bites of vampire bats: 25 out of 55 guinea pigs (45.4 per cent), 3 out of 11 horses and mules (27.3 per cent). One horse and one mule were each infected from a single bite.

7. In the light of our present knowledge concerning the vampire bat as a transmitter of "murrina" under natural conditions, it is recommended that equines be protected from the attacks of bats. Fortunately, protection from bats can be effected with greater ease than is the case with diptera or other arthropods. Adequate protection is provided by stabling the equines from the beginning of dusk to dawn in screened shelters, the wire mesh being not more than one inch, or by illuminating the stables with lanterns or electric light.