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FURTHER STUDIES ON THE TRANSMISSION OF TRYPANOSOMA HIPPICUM DARLING BY THE VAMPIRE BAT DESMODUS ROTUNDUS MURINUS WAGNER[1]

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The disease murrina, an infection of horses due to *Trypanosoma hippicum*, was first recognized in Panama by Darling (1) in 1909. In 1911 (2) he explained the mode of transmission as follows: "The disease is probably transmitted mechanically by flies through the broken skin of cuts and various wounds, and there are no evidences that any animals were infected by means of stomoxys or tabanids or by ticks or bats." No further progress was made in the elucidation of the mode of transmission of this disease until Dunn (3) published his work incriminating the vampire bat *Desmodus rotundus murinus* Wagner, as the probable vector. His results showed that by allowing clean bats to feed on infected animals, they acquired the infection, and in subsequent feeding passed it on to clean animals. He states: "I believe this to be the first time that a biological transfer of murrina has been accomplished, and a true vector identified." Clark (4), in his paper discussing cattle reservoirs of this disease, noted the occurrence of the causative agent *T. hippicum* in the saliva of one vampire bat which was under observation.

This work has been continued principally with the idea of determining how the trypanosomes reach the buccal cavity and in addition to evaluate the rôle of the bats as a vector of the disease. The results, in general, indicate that the bat is not a biological vector and although it does transmit the disease under

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laboratory and box stall conditions its importance in the wholesale spread of it, under natural conditions, is not as great as the many mechanical ways in which fresh infected blood is implanted on recent injuries and open sores.

All the bats used in the experiments were caught in Panama in a radius of 20 miles from Panama City. Some were taken from caves situated on the coast of the island of Taboga, while the others were caught in caves and tree holes on the mainland.

Before the animals were used for experimental purposes blood examinations were made by the thick film technique and by animal inoculations. The bats showing any kind of trypanosome infection were discarded. One bat was found to be naturally infected with *Trypanosoma hippicum* and five were carriers of *Trypanosoma cruzi*. These naturally infected animals will be the subject of other reports.

A total of 39 bats were used and infections were established in all of them. At the beginning of the study infections were produced by allowing the animals to feed on parasitized guinea pigs. When trypanosomes were found in the saliva of these animals, the method of inoculation was changed and the bats were then injected subcutaneously, in order to eliminate the possibility of the parasites localizing in the buccal cavity after an infective meal. Subsequent studies proved this to be a needless precaution.

In Dunn's work (3) the disease was fatal in all cases. This did not prove to be true in the present series. Six bats recovered and are at the present time alive and healthy. Five were inoculated subcutaneously with 0.05 cc. of a mixture of infective blood and normal saline. Four became positive within one week after inoculation and one in two weeks after inoculation. The blood stream infection was never high, 21 trypanosomes being the greatest number found in any thick film examination. The usual number was five per thick drop. In one, trypanosomes were found on two consecutive days and then disappeared. In the other four the infection lasted from one to three weeks, finally disappearing. The sixth bat was one which acquired the infection by feeding. One week after feeding on an infected guinea pig it became positive. In this case the infection lasted about

two months, after that time becoming negative. During the time that it was positive the bat was fed on 8 guinea pigs, 4 of which

TABLE 1

Vampire bats which recovered from T. hippicum infections

BAT NUMBER	DATE OF INFECTION	METHOD OF INFECTION	FIRST POSITIVE	LAST POSITIVE
50	7/30/34	Injected	8/ 2/34	8/24/34
51	7/30/34	Injected	8/ 8/34	8/14/34
54	7/30/34	Injected	8/ 7/34	10/ 8/34
60	7/30/34	Injected	8/14/34	8/15/34
62	7/30/34	Injected	8/ 2/34	8/27/34
73	12/20/34	Feeding	12/27/34	2/11/35

TABLE 2

Showing successful passages by feeding

VAMPIRE NUMBER	METHOD OF INOCULATION	NUMBER OF PIGS USED	NUMBER OF PIGS POSITIVE	PERCENTAGE
V. B. 33	Feeding	5	1	20
V. B. 34	Feeding	3	1	33⅓
V. B. 39	Subcutaneous inoculation	5	1	20
V. B. 38	Feeding	4	1	25
V. B. 42	Feeding	2	0	0
V. B. 44	Subcutaneous inoculation	4	1	25
V. B. 45	Subcutaneous inoculation	3	0	0
V. B. 46	Subcutaneous inoculation	4	1	25
V. B. 47	Subcutaneous inoculation	4	3	75
V. B. 57	Subcutaneous inoculation	4	3	75
V. B. 67	Subcutaneous inoculation	8	2	25
V. B. 68	Subcutaneous inoculation	4	2	50
V. B. 40	Subcutaneous inoculation	1	1	100
V. B. 48	Subcutaneous inoculation	1	1	100
V. B. 56	Subcutaneous inoculation	4	1	25
V. B. 65	Subcutaneous inoculation	8	4	50
V. B. 86	Subcutaneous inoculation	1	0	0
V. B. 73	Feeding	8	5	62.5
V. B. 87	Subcutaneous inoculation	1	1	100
V. B. 88	Subcutaneous inoculation	1	1	100
V. B. 89	Subcutaneous inoculation	1	1	100
Total (21 bats).....		76	31	40.7

became positive. The trypanosomes have never reappeared in the circulation of these 6 bats; numerous blood examinations and

animal inoculations have been made from time to time to check this. In one other bat, discussed by the author in another paper (5), the infection was also aborted. With this bat the infection was not passed to laboratory animals by feeding. This makes 7 bats, out of a total of 50 which have been used at this laboratory for experimental purposes, that have recovered from the infection (see table 1).

Twenty-one of the bats were allowed to feed on clean pigs at various intervals and the results are given in table 2. Eighteen of these succeeded in passing the infection. In each case the pig was used only once for feeding. As can be seen from the table, out of 76 pigs used, 31 or 40.7 per cent became positive. This percentage is slightly lower than that given by Dunn (3). He used 55 pigs and out of this group 25 became infected or 45.5 per cent

TISSUE STUDIES

The tissues of 15 of the bats were examined. The group contained individuals which had been infected both by feeding and by subcutaneous inoculation and all had, at one time or other, shown trypanosomes in their saliva. The animals were killed with chloroform and all the organs removed and fixed immediately. The method of preparation and staining of the tissue was that of Wolbach's (6). Other techniques were tried and discarded as they did not prove successful in our hands. In this technique the tissues were fixed in a corrosive sublimate mixture and subsequently stained with Giemsa.

The tissues were free of lesions and showed only a moderate congestion. In some that had been killed because their condition made it evident that they could not survive for a much longer period, the liver and kidneys showed the usual congestion and in addition a moderate parenchymatous degeneration. The spleens were usually enlarged.

Trypanosomes were found in the blood vessels of all the organs and tissues studied and not infrequently they were seen in the connective tissue adjacent to these structures. In the situations outside of the blood vessels they were never accompanied by any

inflammatory or other type of lesion. No evidence of a cyclic development in the tissue could be found. In the salivary glands they were never present in the ducts and the mucous membranes of the mouths were also always negative, no trypanosomes ever being found in a position which suggested migration into the buccal cavity.

EXAMINATION OF SALIVA

Saliva examinations were made upon the entire series of bats and in each animal trypanosomes were found at one time or another. It was very difficult to make smears from the saliva of these animals without causing bleeding from the membranes. They are very excitable and ferocious and when picked up will struggle and bite fiercely at the glove worn by the person handling them, digging out good size pieces of leather from the glove. We were able to demonstrate trypanosomes in the saliva in only three instances, once in each of 3 bats, in which there was no associated bleeding from the mucous membranes. These 3 cases are given in detail below.

Bat 33. The animal fed on an infected guinea pig on February 12, 1934 and blood films were first positive on February 17, 1934. On February 13, 15, 24, 30 and March 7, the bat was allowed to feed on clean pigs. Examination of the saliva on these dates failed to show trypanosomes except on the last, when they were found unassociated with bleeding. The pig that the bat had fed on became positive and it was the only one that became positive. The bat died five days later without showing any further positive saliva. A careful histological examination of the tissues failed to show any trypanosomes outside of the blood vessels of any of the structures associated with the buccal cavity.

Bat 67. This animal was inoculated subcutaneously with a small amount of infected guinea pig blood on September 24, 1934. The first positive blood smear was seen on September 26, 1934. On October 6, 9, 11, 15, 17, 19, 22 and 24 the animal was allowed to feed on clean pigs. On October 9, the saliva smears were positive for trypanosomes and blood cells, the pig which it fed on became positive. On October 11, the trypanosomes were found in the saliva smears unassociated with blood cells. The pig upon which it fed on this date also became positive.

No further positive saliva was seen and none of the other pigs became positive. The bat died on October 29, 1934, and tissue examinations were negative.

Bat 68. The animal was inoculated subcutaneously on September 24, 1934 and the blood was first positive on September 26, 1934. On October 2, 4, 6, and 9 it was fed on clean pigs. On October 6, the saliva was found to contain trypanosomes and red blood cells and the pig upon which it fed became positive. On October 9, the saliva was again positive but this time no red blood cells were found. The pig used on this date became positive. The bat died on October 10, 1934 and the tissues were negative.

There is only one other recorded case of trypanosomes being present in the saliva of an infected vampire bat unassociated with red blood cells, and that is the one reported by Clark (4).

A word is necessary in regard to the morphology of the trypanosomes that were found in smears unassociated with red blood cells. They were few in number in each of the cases mentioned above and the majority of them showed some morphological variation from the normal. They usually appeared in a rounded up condition with the various structures staining very poorly. Some showed definite evidences of degeneration, with the loss of the flagellum, or kinetoplast or partial extrusion of the nucleus from the body. This degenerated appearance of the parasites suggested a deleterious effect on the part of the saliva. In order to check this point, heavily infected rats were bled to death and the trypanosomes separated from the blood by centrifugation. A drop or two of the concentrated flagellates were then placed in the mouth of a bat and smears were made immediately and at five-minute intervals up to 30 minutes. This procedure was carried out on five clean bats and the same general results were obtained in each case. Between five and ten minutes after the material was introduced into the buccal cavity, rounded up forms, which stained poorly, were found. From ten minutes on these forms became more numerous compared with the number of normal forms seen, and from 15 minutes until the making of the smears was discontinued, breaking up and destruction of the parasites was evident.

Control smears made from the original batch of trypanosomes did not show this phenomena. It was concluded from this that the trypanosomes could not withstand the action of the saliva for a period of more than 25 or 30 minutes. Nevertheless, all 5 of these bats acquired the disease from this experimental administration to test the action of the saliva.

DISCUSSION

The present studies confirm the experimental results of Dunn (3) in that the vampire bat can be infected with *Trypanosoma hippicum* by feeding on parasitized animals and can transmit the disease to clean animals by subsequent biting and feeding.

The infection in the present experiments was not fatal in all cases as was reported by Dunn for his series of animals. Six of the bats recovered and up to the present time have not relapsed.

Trypanosomes were found in the saliva of the entire group of bats used, but with the exception of three instances, one in each of 3 bats, their presence was always associated with red blood cells. It would appear that any minute scratch or injury to the oral mucosa, from which blood could escape would be sufficient to allow the escape of trypanosomes also. The nature of the bat makes the handling of them very difficult and during the process of taking them from their cages and in the subsequent manipulation their struggling and biting often resulted in some injury to the mucous membrane of the mouth, allowing the escape of blood and trypanosomes.

The possibility that the trypanosomes migrate through the oral mucosa has been constantly in our minds and although we feel that the appearance of these parasites in the saliva is dependent upon some injury to the oral mucosa, we have, however, not lost sight of the facts that indirectly there is evidence in favor of this migration. Clark and Darling were successful in infecting a mare by putting blood containing *Trypanosoma hippicum* in the vaginal vault. In another instance infection was brought about by the introduction of infected blood into the nasal passages of a horse. In both instances infection must have occurred because of the ability of the trypanosomes to pass through the normal

mucosa. In the vampire bat the infection is brought about in the same manner, the trypanosomes penetrating the mucosa of the intestinal tract. There is little doubt as to the ability of these parasites being able to penetrate these normal barriers and it seems reasonable to suppose, therefore, that since they can enter the body by this route they should also be able to leave by the same way.

The findings of the trypanosomes in the saliva of bats 33, 67 and 68 might be considered as evidence in favor of the migration of the trypanosomes through the oral mucosa. However, another interpretation is possible. It should be remembered that in bat 33 feeding had occurred on four previous occasions, during which time injury to the mouth might have occurred. In the case of bats 67 and 68, feeding had taken place two days previous when trypanosomes and erythrocytes had been found, establishing the fact that injury to the mucosa had taken place. It follows that the injuries in all 3 cases might have been of sufficient severity that serum and trypanosomes could ooze out for some time before healing was completed.

We feel that as a factor in the rapid spread of the disease in a herd that the vampire bat is of less importance than ordinary mechanical means. In the actual spread of the disease, during epidemic periods, Clark (7) feels that any mechanical means, that can apply fresh wet blood in a fair amount from infected animals to open moist wounds in the skin or mucous membranes of another animal, may transfer the disease. In his opinion an important mechanical means of transfer is caused by the crowding and rubbing together of animals under excitement such as being driven in from pasture and held in corrals. Such animals in the tropics have myriads of insect bites, thorn punctures, grass and wire abrasions, etc.

Where the bat does feature as an important vector is during the periods between epidemics when the disease is present mainly in cattle and perhaps a few chronic carriers among horses and mules. They can pick up the infection from carriers and convey it to new herds. It is quite possible that to a certain extent they act as a reservoir themselves, in as much as six of the present group have

overcome the infection. One bat showed trypanosomes in its blood for a period of two months before they disappeared.

The bats feed nightly and if unmolested will lap blood for an hour or two. One was actually seen to feed for a period of two hours and forty minutes. Under these conditions there is opportunity for them to acquire the infection, if the trypanosomes are few in number as in cattle carriers and in subsequent feedings pass it on. They do not always become infected from these light cattle carriers, but what is certain is that the number of times they feed and the length of the time of feeding enhances the chances of becoming infected and of transferring it.

The vampire bat in view of the studies presented cannot be considered a biological vector, at least not in the sense that a mosquito is a vector of malaria. It must be considered a mechanical vector but quite different from what is usually meant by mechanical conveyors because of the fact that it actually acquires the disease itself and as long as it has it, is able under certain conditions to transmit it.

The transmission of *Trypanosoma cruzi* by these bats has been attempted six times, by allowing them to feed on pigs, but the results have been negative. One attempt was made to pass the spirochete of relapsing fever, but this was likewise negative. Further work must be carried on in these two diseases before a final conclusion can be reached.

CONCLUSIONS

1. The vampire bat is not a biological vector of *Trypanosoma hippicum*. It is considered to be a mechanical vector of the disease.

2. The trypanosomes reach the saliva of the bat through breaks in the oral mucosa, and possibly by migration through the intact mucosa.

3. The saliva is deleterious to the trypanosomes.

4. The infection in the bat is not always fatal. Six recovered and have not yet relapsed.

5. Tissue studies failed to show the trypanosomes in the lumen

of the ducts of the salivary glands or in any other structures closely associated with the oral cavity.

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DISCUSSION

Dr. Herbert C. Clark: Experimental circumstances under which we have established *T. hippicum* infections, apparently, through normal tissue barriers are as follows:

1. By introducing a large amount of infected blood into the vaginal vault of a mare.
2. By introducing infected blood into the nasal cavity of a horse.
3. Six dogs were allowed to feed on fresh muscle and liver from a freshly killed infected horse. One dog acquired the disease.
4. A vampire bat was fed on a clot of blood about one hour after the blood was taken from the vein of an infected horse and it acquired the disease.

I think this evidence shows that this trypanosome can penetrate normal tissue barriers even though we have been unable to demonstrate it in microscopic examinations of the sectioned tissue.

Vampire bat feedings under natural conditions on a range are apt to occur nightly over long periods of time on the same collection of animals and many times a cluster of bats will be found feeding on one animal. This offers a better chance for acquiring and transmitting the disease

than one or two experimental feedings in a laboratory. Many times the bats do not make a new incision of the skin to feed, they will feed in numbers about the margin of large sores.

The vampire bat and cattle carriers can maintain the disease in a region long after all equines are killed or cured following an epidemic. They can start the disease in a new herd even though the rapid spread of the disease may be due to many other mechanical factors. Coition is an important factor in a breeding herd at large on unfenced ranges. One infected stallion can conceivably infect many mares. In my opinion, this disease could rapidly be controlled if the ranges were fenced so that horses and mules could regularly be inspected and cattle carriers removed. I consider the vampire bat as an important method of transfer from cattle carriers to new herds of equine stock.