

## ATTEMPTS TO TRANSMIT TRYPANOSOMA CRUZI CHAGAS WITH TICKS OF THE GENUS ORNITHODOROS

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In 1912, Brumpt (1) while conducting studies on the development of *Trypanosoma cruzi* Chagas in various sanguivorous arthropods found that this trypanosome is capable of development in the African tick, *Ornithodoros moubata*. In 1914, Mayer and Rocha Lima (2) while working along similar lines also obtained development of this trypanosome in the same species of tick. They did not, however, succeed in transmitting the infection through the bites of this tick. A later observation by Mayer (3) demonstrated that the infection of *T. cruzi* persisted in the intestinal tract of *O. moubata* five years after the tick had fed on the infected animal.

In view of these results obtained with *O. moubata* and since human trypanosomiasis, or Chaga's fever, the condition produced by infection with *T. cruzi*, is present in Panama it was decided to attempt some transmission experiments with the two species of *Ornithodoros*, *O. venezuelensis* and *O. talaje*, found in this part of tropical America.

In order to avoid unnecessary repetition of words in connection with the mention of each animal used in these experiments, I will here take the opportunity of explaining the method of making these observations. Guinea pigs were used for the experimental animals. In the tick feeding tests an extensive area on the belly of each animal was shaved. The ticks to be fed were placed in straight-walled glass tubes, 1 by 4 inches, and the latter inverted on the shaven area of the guinea pig. From four to ten ticks were placed in each tube at a time. The tubes were held by hand on the animal after the latter had been immobilized by being

bandaged to a slender piece of wood. The tubes being 1 inch in diameter were large enough to permit the ticks to move about to a slight extent on the skin of the animal to include the potential contamination of the lesions caused by the bites of the ticks with the coxal fluid of the latter. The ticks were allowed to remain on the animal as long as they would take blood. Each lot of ticks injected was macerated in from 0.5 to 1 cc. of normal saline solution and the resultant suspension injected into the peritoneal cavity of the animal. A daily examination was made of the blood of each experimental animal for a period of at least two months after being used, with the exception of those that became positive earlier. Thick drop films were used in all the blood examinations. The approximate number of trypanosomes present in a film or microscopic field of blood of each infected animal on which the clean ticks were first fed has been given in order to show how heavily each animal was infected at the time the ticks were fed. The number given for a film signifies the number of trypanosomes found during a complete search of a thick drop film of blood about 14 mm. in diameter. In cases of heavier infection the number counted in one field of the microscope, when viewed with a  $\frac{1}{4}$  inch oil immersion objective and a 6 X ocular, is given.

#### EXPERIMENTS WITH ORNITHODOROS TALAJE

*Experiment 1.* On April 7, 1931, a lot (no. 1) of 39 adult ticks, *O. talaje*, were fed on guinea pig 62 which was infected with *Trypanosoma cruzi*. The blood of this animal showed about two trypanosomes in each microscopic field at this time. On May 15 these 39 ticks were fed on clean guinea pig 72. On July 23 and 24, 36 of these ticks were fed on clean guinea pig 146. The latter animal died on July 26, which was too early to allow us to ascertain the result from the bites of the ticks. On August 25, 25 of the males in this lot of ticks were macerated and injected into clean pig 82. On August 26, 6 large female ticks of this lot were fed on clean pig 163. On December 19 the same 6 females were fed on clean guinea pig 246. Each of these ticks became well engorged and each secreted coxal fluid which was rubbed into the

lesions, made by the bites, with a platinum loop. On January 14, 1932, these 6 females were macerated and injected into clean guinea pig 269. No results were obtained in this experiment.

*Experiment 2.* On April 9, 1931, a lot (no. 2) of 28 adults of *O. talaje* were fed on infected guinea pig 71. The blood of this animal was showing about five *T. cruzi* in each microscopic field at the time the ticks were fed. On May 16 these 28 ticks were fed on clean guinea pig 73. On July 30 and 31, 28 of them were fed on clean guinea pig 147. This second animal died on August 3, which was too soon after the feeding to ascertain the results. On December 17 and 18, 25 of these ticks, 13 females and 12 males, were fed on clean guinea pig 243. All of the females and 5 of the males secreted noticeable amounts of coxal fluid. On January 19, 1932, 8 females of this lot were fed on clean guinea pig 273. On February 9 the 13 females and 12 males remaining in this lot were injected into two clean guinea pigs, 125 and 289. None of the animals used in this experiment became infected.

*Experiment 3.* On October 8 and 9, 1931, a total of 70 second stage nymphs of *O. talaje* were fed on infected guinea pig 169. At this time the blood of the animal showed about 175 *T. cruzi* in a thick film. All these ticks became engorged. On November 4, 38 more second stage nymphs were fed on another infected guinea pig, no. 188. There were about two *T. cruzi* present in each microscopic field of a blood film from this animal at the time of the feeding. These ticks all became engorged and were then added to those that had fed on October 8 and 9 and the combined lots designated as lot 3. These ticks molted to third stage nymphs and on December 10 and 11, 100 of them were fed on clean guinea pig 237. Practically all became well engorged and secreted coxal fluid. These ticks again molted and became fourth stage nymphs and on February 12 and 13, 1932, a total of 96 of them were fed on clean guinea pig 296. On February 29, 95 of these ticks were macerated and injected into clean guinea pigs 207 and 252. No results were obtained in this experiment.

*Experiment 4.* On September 28, 1931, a lot (no. 4) of 21 female ticks, *O. talaje*, were fed on infected guinea pig 169. This guinea pig was showing about 24 *T. cruzi* in a thick drop film of

its blood at this time. All the ticks became engorged. On October 19 and 20 these 21 ticks were placed on clean guinea pig 202. All fed and became engorged and each secreted some coxal fluid. On October 22 and 23 another lot (no. 5) of 21 male ticks were fed on the infected guinea pig 169, which was then showing about 84 trypanosomes in a film of its blood. On November 30 and December 1, 20 of these male ticks were fed on clean guinea pig 231. On December 2 and 3, 16 of the females of lot 4 were also fed on this animal. On January 14 and 16, 16 of the females and 17 males were fed on clean guinea pig 270. Since the ticks of lots 4 and 5 were part of a number being observed for life history studies they were not macerated and injected. This experiment gave no results.

#### EXPERIMENTS WITH ORNITHODOROS VENEZUELENSIS

*Experiment 1.* On December 20, 1930, a total of 58 *O. venezuelensis* adults were fed on infected guinea pig 22. The blood of this animal was showing about one *T. cruzi* in each microscopic field at this time. Only 16 of these ticks were alive February 2 and on that date these survivors were fed on clean guinea pig 36. On April 16, 12 of these ticks were again fed on this same animal. On May 4, these 12 ticks were macerated and injected into clean guinea pig 036. This animal became positive, with trypanosomes appearing in its peripheral blood on May 26. No results were obtained from the bites of the ticks.

*Experiment 2.* On December 27, 1930, a total of 31 adult *O. venezuelensis* were fed on infected guinea pig 22. *T. cruzi* were numerous in the blood of this animal at this time, as many as 16 being present in a microscopic field. On April 11, 12 of these ticks were fed on clean guinea pig 91. On May 6 these 12 ticks were injected into clean guinea pig 091. This animal became positive on May 18. No results were produced by the bites of the ticks.

*Experiment 3.* On January 8, 1931, a lot (lot C) of 22 adult *O. venezuelensis* were fed on infected guinea pig 27. The blood of this animal showed about two *T. cruzi* in each microscopic field at this time. On February 21, 13 of these ticks were fed on clean

guinea pig 64. On April 16, 11 of these ticks were again fed on this animal without results. On May 5, 10 of these ticks were injected into clean guinea pig 064 with positive results. Trypanosomes appeared in the blood of this animal on May 20.

*Experiment 4.* On September 16, 1932, a number of larvae of *O. venezuelensis* were fed on infected guinea pig 421, which was showing about eight *T. cruzi* in each microscopic field of its blood at this time. As these ticks later molted and advanced to more mature stages they were fed on a clean guinea pig in each stage as follows: 16 first stage nymphs on animal 477 on October 21, 13 second stage nymphs on animal 486 on November 4, 13 third stage nymphs on animal 492 on November 21, 16 fourth stage nymphs on animal 502 on December 9 and 15 adults were fed on animal 536 on January 4, 1933. There were no results from this experiment. Since these ticks were also being used for biological studies on this species they were not killed and injected.

*Experiment 5.* On September 14, 1932, a total of 28 larvae of *O. venezuelensis* were fed on infected guinea pig 421. Thick drop blood films from this animal showed about five *T. cruzi* in a microscopic field at this time. As in experiment 4, these ticks were fed on a clean guinea pig each time they molted and advanced to a more mature stage. Thus five animals were used with an interval of from two to four weeks elapsing between each feeding. The fifth feeding consisted of 20 adults that were fed on animal 526 on December 30. On March 17, 1933, there were 7 adult ticks of this lot still alive and on that date they were fed on clean guinea pig 512. On March 20 a leg was pulled from each of these ticks and a small smear made from the fluid that exuded from the stump of the leg on each tick. Three of these ticks apparently were injured during the removal of the legs to such an extent that rupture of the intestines occurred and some of the intestinal fluid mixed with coelomic fluid and thus became present in the smears. Three trypanosomes were found in one of the smears in which some of the intestinal contents were present. None were found in the other six smears. After obtaining the smears from these ticks the latter were then macerated and injected into two guinea pigs. One of these, no. 335, was injected with three ticks, one

male and two females. The second animal, no. 336, received four ticks, three males and one female. Both of these animals became positive. Trypanosomes appeared in the blood of animal 336 on April 3 and in animal 335 on April 5. None of the animals on which the ticks were fed became positive.

*Experiment 6.* This experiment was similar to the preceding one. On October 7, 1932, 36 larvae of *O. venezuelensis* were fed on infected guinea pig 432. This animal showed about five *T. cruzi* in each microscopic field of its blood at this time. As in experiment 5, these ticks were later fed on a clean guinea pig after each molt, thus being fed on five animals during the three months subsequent to their feeding on the infected guinea pig. On March 20, 1933, there were 6 of these ticks still alive and these were again fed on a clean animal. After these ticks finished feeding this time a leg was pulled from each and smears made from the fluid that exuded. Ten trypanosomes were found in one of these smears. No feces could be detected in this smear. The other five smears were negative. These ticks were then injected into two clean guinea pigs, nos. 446 and 449. Four ticks, two males and two females, were injected into animal 446 and one male and one female into animal 449. Both these animals became positive on April 3 after an incubation period of fourteen days. No results were obtained from the biting tests.

#### SUMMARY AND CONCLUSIONS

Attempts to transmit *Trypanosoma cruzi* by means of two species of ticks, *Ornithodoros talaje* and *O. venezuelensis*, were undertaken as follows:

Four lots of *O. talaje* were fed on guinea pigs infected with *T. cruzi* and subsequently fed on 15 clean guinea pigs. Two of these latter animals died too early for the results to be determined but the other 13 remained negative. Some of these ticks were later macerated and injected into 6 clean animals but this procedure also failed to produce any infection. These negative results would indicate that this species of tick does not commonly transmit *T. cruzi* by means of its bite and also that if the trypanosome undergoes any development in this tick the infection in the latter does not persist for a very long period.

Several lots of immature and adult *O. venezuelensis* were fed on guinea pigs infected with *T. cruzi* and later fed on 20 clean guinea pigs. Three of these latter animals were hosts either for one lot of the ticks on two occasions or for two or more lots. No results were obtained from these ticks feeding on the animals. Some of these ticks were later macerated and injected into 7 guinea pigs and each animal became positive after incubation periods of from fourteen to twenty-two days. These results tend to show that although *T. cruzi* may develop and persist in *O. venezuelensis* for a period of more than six months this tick does not usually transmit the infection to its hosts through feeding on them.

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