EQUINE TRYPANOSOMIASIS—MURRINA OF PANAMA

HERBERT C. CLARK, *Director, Gorgas Memorial Laboratory, Panama, Republic of Panama*

The first scientific record in regard to the presence of equine trypanosomiasis on the Isthmus of Panama was made by Darling (1) in 1909, and the report was published in 1910. The disease has made great inroads upon the horse and mule population in the past and is still a serious problem in the cattle ranches where saddle ponies and pack horses are a necessity.

A similar, if not identical disease has been known for over 100 years in Venezuela and Colombia. Rangel (2) in 1905 discovered the parasite and Mesnil (3) in 1910 named it *Trypanosoma venezuelense*. Iturbe (4) reported their ideas in 1924 on the general character of the disease and its management. Rangel claimed that the disease presents two different clinical aspects: the *Peste boba* and the *Derrengadera*. Enormous losses have occurred in the cattle plains of Venezuela. Our observations over the past years in Panama indicate that *Peste boba* represents the acute period and *Derrengadera* the late and chronic stages of the same disease.

ANIMAL SUSCEPTIBILITY TO TRYPANOSOMA HIPPICUM

Iturbe reports that the disease has been found spontaneously in certain animals peculiar to Venezuela and Colombia. He mentions that it can frequently be found in mules and asses, in dogs, chigüires (*Hydrochoerus capivara*), foxes (*Canis azarae*), and in large monkeys called araguatos (*Micetes ursinus*). Our own experience in Pamana since 1909 has not revealed spontaneous infections in any animals except horses, mules, asses, cattle, and dogs, and we have seen only three dogs with the spontaneous infection even though we have examined many that lived with herds of horses on the ranches.

Animals that can be infected in our region are listed by Clark and Dunn (5), but for laboratory use the guinea pig, the rat, the mouse, and the dog are recommended. The capybara (*Hydrochoerus isthmius* Goldman) or poncho is not found in Panama except in the Tuira River Valley next to Colombia. Three of them were brought to the laboratory in Panama City. Two were given a strain of *T. hippicum*. Both developed the disease and died, one in 15 days and the other in 20 days. The third animal was not inoculated and lived with the other two. It did not contract the disease. We consider the capybara the most susceptible animal we have studied, and it makes a splendid animal in which to develop an abundance of the parasites. These can then be collected to serve as an antigen.

Clark and Dunn (5) examined the following wildlife in Panama for naturally acquired infections of *T. hippicum;* deer (2 species),

wild hog or peccary, wild rats and mice, squirrels (2 species), armadillo (2 species), sloths (2 species), porcupine, agouti (2 species), opossums (3 species), coati, tapir, capybara, bats (6 species), monkeys (10 species), and domestic chickens, ducks, and turkeys. We were never successful in our efforts to infect poultry. Only a rare vampire bat was ever found naturally infected, but all the other animals mentioned were susceptible to laboratory inoculations.

We were able to examine eight tapirs (*Tapirella bairdii* (Gill)). This animal is the closest relative to the horse of any wild animal in the Western Hemisphere. None of these eight animals revealed trypanosomes in blood films. All were infants except two that were killed in the jungle and could not be brought to the laboratory. Blood films from these were negative. Only one infant tapir reached our laboratory in apparently satisfactory condition to study. It was examined daily for trypanosomes from September 21 to 30, 1931, with negative results. It was inoculated on September 30, 1931, and *T. hippicum* appeared in the blood films on October 2. It died on October 12. This is a short course for the disease. Fright from capture, transportation, and captivity, as well as separation from the mother may have been contributory causes of death.

Animals that may be infected and live for weeks or months are the mule, ass, horse, domestic cat, white-tailed deer (*Odocoileus chiricuensis* Allen), brocket deer (*Mazama sartorii reperticia* Goldman), and the wild hog or collared peccary (*Pecari angulatus bangsi* Goldman).

Animals that are susceptible of infection and can carry the infection for a long time without symptoms and then recover spontaneously are cattle, domestic hogs, sheep (Algerian breed), and native goats.

Two accidents involving human beings indicate that *T. hippicum* will not infect man. One was in a Negro and the other in a white man. Each had a syringe of blood just drawn from the jugular vein of an infected horse and was about to inject several guinea pigs with the blood when they were each kicked on the arm, the syringes were shattered, and the fragments driven into the palms of the hands. In one case, the needle also passed into the deep tissues of the palm. Although in each case an effort to sterilize the wounds was made, it was quite impossible to treat the deep, penetrating wounds. Neither man ever showed a parasite.

MODE OF TRANSMISSION

The method of transmission has always been the most important problem in our local studies since Darling found the parasite. In 1912 Darling (6) succeeded in infecting a mule by allowing 18 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 a

mule. While this transmission may be termed a purely mechanical one, it was the only time that positive results had been obtained by using insects (flies, ticks, mosquitoes, etc.). We know that a mass of flies, ticks, or mosquitoes recently fed on highly infected blood can be crushed and rubbed into the skin and occasionally produce a positive result. Iturbe (4) reports success in transmission by injecting the intestinal contents of a gad-fly (*Tabanus importunus* Wied.).

Any mechanical means that can apply fresh blood in a fair amount from an infected animal to an open moist wound in the skin or mucous membranes of another animal may in some instances transfer the disease. It can pass through the mucosa of the mouth, vaginal vault, etc., if heavily infected blood can be introduced and retained. An important mechanical means of transfer could be the crowding and rubbing together of untrained, wild horses under the excitement of being driven into corrals for branding purposes. They always crowd into a corner, and both sides are rubbed hard against the neighboring animals.

All animals on pasture are sure to have innumerable insect bites, thorn and wire injuries, harness and saddle galls, or rope burns. Insect vectors can transmit by mechanical means, but circumstances at present do not indicate that any of them are important hosts for the parasite for more than a day or two. For years we have seen well-groomed animals in unscreened but illuminated stables; and unless they are taken into endemic areas for periods of time, they seldom acquire the disease. This seems to offer proof that flies, mosquitoes, and ticks are not the common cause of the spread of the disease. The fact that certain flies in eastern countries have been incriminated as vectors of trypanosomal diseases and that vampire bats do not occur in those regions of the Far East where one form of equine trypanosomiasis is endemic, had kept us for years away from studies of our local vampire bats. The bat is a mammal, which also made it seem very unlikely that it might be a vector. However, in 1929 and 1930 we began some studies on the bat, and in 1932 Dunn (9) reported the vampire bat, *Desmodus rotundus murinus* Wagner, to be a vector of the disease. The incubation period in the bat is from 6 to 8 days. It is fatal for the bats, in most instances, within a month, although a few have survived and spontaneously recovered. The appetite of the bat is not impaired by the disease, so there is ample time and opportunity for it to spread the disease before it dies. In five experiments with positive results, the following clean animals became infected through the bites of vampire bats: 25 of 55 guinea pigs, 3 of 11 horses and mules. One horse and one mule were each infected from a single bat bite. We have no way of measuring the size of the blood meal taken by a vampire bat when it feeds under normal conditions and becomes distended, but we have learned to keep and feed them in captivity and under such circumstances they average 16 cubic centimeter of defibrinated blood each night. Naturally such

a meal makes it possible for them to infect themselves even when feeding on a light carrier of the trypanosomes.

Contrary to the opinion that most people seem to have, the vampire bat does not suck blood. It laps up the blood just as a cat laps up milk. It does this very fast; and if the wound it makes does not bleed freely, it renews its bite to produce better results. Nearly everyone overlooks many of the bites on stock caused by vampire bats, because most of their bites do not continue to bleed and run through the hair to form a cord of clotted blood, such as one sees on the withers of horses and mules. We have been able to observe their habits in captivity and to some extent outside captivity. In a screened box stall with a concrete floor, they frequently elect to feed at the hair line on the back of the front foot. The horse makes no attempt to dislodge them. At other times the bat may be compelled, because of the position of the horse, to select a site with long thick hair, and when this happens it spends considerable time clipping away the hair until a spot of a quarter to a half-inch in size is denuded before it bites the skin. It is easier to lap up the blood from such a denuded spot. A mother bat carries her young across her chest until it is nearly as large as she is before it is weaned, and we have once observed three mothers with their young all feeding at the same time on blood oozing from the edge of a large sore on the shoulder of a horse. We know from studies made on bats in captivity (8) that 27 noninfected vampire bats have lived from 5 to 13 years in our animal house. We do not know the age of any of them when they were first placed in captivity. They can make long flights if necessary; but if cattle and horses are present and a refuge is available, they do not range very far. Caves, hollow trees, and thatched roofs make a good refuge for them. Seven vampire bats of a large number captured in a hollow tree in the center of a large cattle region near Pacora, Republic of Panama, were found to have naturally acquired infections of *T. hippicum*. These strains were used on guinea pigs and a horse with successful results. Fortunately animals can be protected from bats if it is possible to keep them in screened stables or in stables illuminated during the night. Cattle, horses, and mules that are kept in pastures day and night cannot be protected.

Clark and Dunn (9) had an opportunity to study 135 head of cattle taken from an area where murrina was endemic. These animals were slaughtered in a Canal Zone packing house, and we examined thick blood films and inoculated guinea pigs with from 2 to 4 cubic centimeters of jugular vein blood. None of the blood films revealed trypanosomes, but 4.5 percent of the guinea pigs developed *T. hippicum* infections. We learned later that some of these cattle came from Venezuela, so this study was repeated on cattle known to have been born and raised in Panama (10). The Province of Panama had no positive cattle in 71 examined, Coclé had 4 positive in 148 cattle tested, and Herrera had 1 positive in the 15 tested. Thus 234 cattle from endemic areas showed 5, or 2.1 percent, positive for *T. hippicum*. Native cattle

of the Republic of Panama can act as an important unharmed reservoir for *T. hippicum*, the cause of equine trypanosomiasis. Various cattle areas in Panama show that from 2 to 6 percent are carriers wherever the horse disease has been endemic. Three vampire bats were fed on cattle carriers. One of the three acquired the infection and transferred it to a guinea pig. Where cattle and horses range together without protection at night from vampire bats, the question of control becomes a serious problem. We think it safe to assume that the animal reservoirs of greatest importance for the perpetuation of *T. hippicum* in Panama are the horse, mule, ass, and cattle. Those of secondary importance are hogs, deer, sheep, goats, and domestic dogs and cats.

CLINICAL PICTURE OF THE DISEASE

The first cases that appear in a herd are apt to escape attention since it requires a period of a few weeks for the disease to develop significant clinical evidence and a few months for it to gain an extensive spread in a herd of animals. The spread is far more rapid in herds on pasture than in animals kept in stables.

The clinical picture is marked by fever of an irregular character, progressive emaciation, anemia, faint icterus, a rough coat, and frequently edema of the most dependent part of the abdominal wall and sheath. Late in the illness there is a marked weakness of the posterior extremities, and the animal walks with a stiff, staggering gait, sometimes dragging the point of the hoof. The odd feature is the fact that there is no impairment of the appetite throughout the course of the disease. The clinical picture is presumptive evidence of the disease, and laboratory tests are indicated.

Diagnosis can be made by the use of blood film examinations for the detection of the trypanosomes, inoculations of any susceptible animals (usually guinea pigs), or the application of the complement-fixation test for equine trypanosomiasis. Fresh blood films can be made in the field and searched for the parasites if portable equipment is available. We find it more satisfactory to use the thick-blood film of Barber and Komp (11). A herd should be examined every 2 or 3 days for at least 10 surveys in order to pick out all infected animals. Some prefer to send sera to a government laboratory where a complement-fixation test, as recommended by Mohler (12) for dourine, can be performed. Animal inoculations, using 2 to 4 cubic centimeters of blood by the intraperitoneal route, may succeed at times where other methods fail. It will soon be possible to segregate all the infected equine stock.

It is a fatal disease in almost every case unless treatment is instituted in the very early stage of the disease.

No entirely satisfactory treatment is available, but the best at the present time seems to be the use of Bayer 205 given with tartar emetic. It has been our experience that if one succeeds in curing an animal

in the late stage of the disease it is too stiff and crippled ever to be of much service.

Darling has recorded the pathological anatomy in his early reports. We consider it difficult to establish a diagnosis at autopsy unless *T. hippicum* can be found in the blood or tissue films.

Infected guinea pigs give birth to healthy offspring, and these can be given the disease and killed by it in the usual time of 4 to 8 weeks.

It was possible in one epidemic to study 27 mares and their foals with the following results:

Dams positive for the disease and foals negative_____ 14
Dams negative and foals positive_____ 9
Dams and foals both positive_____ 4

One horse treated in the late stage of the disease was apparently cured. Some months later it was experimentally reinfected and died of the disease.

We carry two strains of *T. hippicum*. One killed 33 guinea pigs in an average of 31 days, and the other killed 36 guinea pigs in an average of 37 days.

DESCRIPTION OF *T. hippicum*

The typical parasite, according to Darling, is 16μ to 18μ in length and 2μ wide. The distance from the kinetoplast to the posterior tip is 1.75μ, and the distance from the posterior tip to the middle of the nucleus is 7.5μ to 10μ. The flagellum is usually short and frequently not entirely free, for often the attenuated process of the cyptoplasm extends to the extreme end of the chromatin filament. Sometimes there is a free flagellum reaching 4μ in length. The posterior tip of this trypanosome is rather blunt. The cytoplasm usually contains scattered basophilic granules, and the majority of these are in the anterior half of the parasite. A well-developed undulating membrane is present.

Darling believed it evident that this parasite was different from *T. equinum* and *T. equiperdum*. He presented material to Laveran and asked for his opinion. Laveran agreed with Darling. Wenyon (14) believed that *T. hippicum* is quite similar to *T. venezuelense* and *T. evansi;* Johnson (15) believed that *T. hippicum* and *T. venezuelense* are the same but did not feel that they are strains of *T. evansi*. He thought that they should be included in the *congolense* group of pathogenic trypanosomes rather than in the *evansi* group.

GEOGRAPHIC DISTRIBUTION AND DIRECTION OF SPREAD

This disease seldom gained a foothold on the west side of the Canal Zone during the building of the Panama Canal. Trails and road systems have now opened for use through the western provinces of Panama, and these have already permitted the disease to become established about 200 miles beyond the west side of the Canal Zone. These roads

will soon form a segment of the international highway; and when this is in operation, there is no reason why this form of trypanosomiasis should not find its way by relay through Central America. We have had opportunities to survey large herds of stock in the Atlantic coastal plains of Costa Rica, Honduras, and Guatemala, but no evidence of the disease has been found. At present the north coast of South America and Panama seem to be the endemic areas.

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