Equine Trypanosomiasis—"Murrina" or "Derrengadera"

Some Notes on the Disease in Panama

By HERBERT C. CLARK, *Director,*
Gorgas Memorial Laboratory, Panama, Republic of Panama,
TIMOTHY L. CASSERLY,
Supply Department of the Panama Canal, Balboa Heights, Canal Zone, and
Major I. O. GLADISH, *V. C., U. S. A.,*
Panama Canal Department, Corozal, Canal Zone

EQUINE TRYPANOSOMIASIS-"MURRINA" OR "DERRENGADERA"

Some Notes on the Disease in Panama*

By HERBERT C. CLARK, *Director,*
Gorgas Memorial Laboratory, Panama, Republic of Panama,
TIMOTHY L. CASSERLY,
Supply Department of the Panama Canal, Balboa Heights, Canal Zone, and
Major I. O. GLADISH, *V. C., U. S. A.,*
Panama Canal Department, Corozal, Canal Zone

The first scientific record made in regard to the presence of equine trypanosomiasis (murrina or derrengadera) on the isthmus of Panama was written in 1910, by Darling.[1] He established the fact that it was a trypanosomiasis and named the pathogenic agent *Trypanosoma hippicum.* The typical parasite, according to Darling, is 16 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 7.5 to 10μ. The flagellum is usually short and frequently not entirely free, for often the attenuated process of the cytoplasm extends to the extreme end of the chromatin filament. At times 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 granules are in the anterior half of the parasite. A well-developed undulating membrane is present.

It is well known that there may be variations in the morphology of trypanosomes especially during the rapid development of a newly acquired infection and also in the very late stage of the chronic infection in resistant animals. Darling[2] believed it quite evident that this parasite was different from *T. equinum* of mal de caderas and *T. equiperdum* of dourine. Laveran[3] expressed the opinion that *T. hippicum* differed from those just mentioned since it possessed a centrosome, basophilic granulations, and a short flagellum. Wenyon[5] believes that *T. hippicum* is quite similar to *T. venezuelense* and *T. evansi.* We think now that he may be justified in holding this view. The clinical picture of this disease of solipeds had been known, before Darling's report on the subject, as "murrina" or "derrengadera" by the people of Panama. It has always been considered as uniformly

*Received for publication, November 29, 1932.

fatal to horses and mules. The course of illness ranged from a few weeks to several months. According to our personal experience it runs a shorter course in the horse than in the mule. The burro is the soliped with the greatest resistance to the disease. This little animal seldom acquires it but when infected it becomes an important reservoir for the trypanosome over a long period of time.

Clinical Diagnosis

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 of the disease is far more rapid in herds on pasture than in a herd kept in stables.

The clinical picture is exhibited by fever of an irregular character, progressive emaciation, anemia, faint icterus, rough coat and sometimes edema of the most dependent part of the abdominal wall and sheath and to a less extent of the legs. Late in the illness there is a marked weakness of the posterior extremities and the animal walks with a stiff, staggering gait, frequently dragging the hoof. There is no impairment of appetite throughout the course of the disease. An emaciated animal with fever, a staggering gait, and a good appetite is strong presumptive evidence of this disease and laboratory tests are indicated. These may consist of blood-film examinations for the detection of trypanosomes, inoculations of susceptible animals and the application of the complement-fixation test for equine trypanosomiasis. We select the thick-blood-film method of Barber and Komp[4] for rapid field surveys, since it is difficult to examine fresh blood-films in the field in cloudy, rainy weather. A herd should be examined every two or three days for at least ten surveys in order to pick out the infected cases and, when possible, it is well to apply the complement-fixation test, as recommended by Mohler[5] for dourine. Animal inoculations using 2 to 4 cc of blood by the intraperitoneal method may reveal, at times, a case that these methods have missed but the combination of the thick-blood-film and complement-fixation tests at intervals will soon make it possible to segregate all of the positive animals.

Pathology

Darling[2] has recorded the pathological anatomy in his report but we consider it difficult to establish a diagnosis safely at autopsy unless trypanosomes can be found in the blood or tissue

films made at the time of autopsy. The lesions described for the disease afford supportive evidence of great value but no gross lesions are absolutely characteristic of this disease in the absence of films positive for the trypanosome.

Mode of Transmission

Dourine[5] is described as a disease of breeding animals and is transmitted from animal to animal by coition, while other means of transmission are considered so rare that they have no practical importance in measures for suppressing the disease. Murrina, our local equine trypanosomiasis, can be transmitted by coition but it differs from dourine in being spread also by other means. Any mechanical means that can apply fresh wet blood in a fair amount from an infected animal to an open moist wound in the skin or mucous membranes of another animal may transfer the disease. It can pass through the normal mucosa of the mouth, vaginal vault, etc., if heavily infected blood can be introduced and retained. In our 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 pastures and caught up in corrals. Animals in this region are covered with innumerable tiny tick and fly bites, thorn and wire injuries, bat bites, harness and saddle galls or rope burns that ooze. Such animals, when they contract the disease, can transfer it by brushing against the sides of negative animals that contain many oozing small wounds. Insects may carry infected fresh wet blood to harness and saddle galls, rope burns, etc., on uninfected members of a herd.

A breeding herd consisting of horses, mules and burros did not show a limitation of the disease to brood mares and stallions. Four colts from eight to ten months of age and two mule colts of the same age were victims of this disease. Nine young geldings and twelve young mares as well as six old mules contracted the disorder. The two stallions belonging to this herd were kept in stables while the other animals were kept in four separate pastures. It is interesting to note, however, that 30 out of 47 old brood mares and one of the two stallions had the disease.

The Supply Department of the Panama Canal, a few years ago, purchased 127 brood mares for the Miraflores Farm from a ranch near Divalá in the province of Chiriqui, Republic of Panama, but this disease has never appeared there according to histories given us by local owners and our recent survey of that region (858 horses and mules), as well as two former

surveys, failed to locate a case of the disease. Thirty of these brood mares acquired the disease on the Miraflores Farm. The stallion found positive at Miraflores was imported from France via Venezuela.

The Vampire Bat as a Vector of the Disease

Dunn,[6] in 1932, discovered from his experimental work on some local species of bats that our vampire bat, *Desmodus rotundus murinus* Wagner, could acquire the disease by feeding on infected animals. He also obtained positive results in five out of six experiments in the transmission of *T. hippicum* to horses. mules and guinea pigs through the bites or feedings of these infected vampire bats. The incubation period in this bat is from six to eight days. The disease proves fatal to the bats in from nine to 27 days after their blood-films reveal the trypanosome. The appetite of the bat is not impaired by the disease and since the animals feed every night and live usually for about a month after becoming infective, there is ample opportunity for them to spread the disease. One of these bats, if unmolested, will feed from one to two hours, voiding urine at frequent intervals. They will average 16 cc of defibrinated blood at a meal if deprived of their natural blood-meal from live animals. This should make it possible for them to infect themselves from relatively light carriers of the parasite. Fortunately, protection from bats can be effected with greater ease than is the case with diptera or other arthropods. Adequate protection can be provided by illuminating the stables either by electricity or by clean oil-lanterns. The owner of a large herd of range animals that cannot be stabled over night is confronted with the more serious problem of keeping the index of infected animals as low as possible to avoid infection of the local vampire bats.

The Question of Insect Vectors

We know that flies can transmit this trypanosome by mechanical means and that blood-meals secured from infected animals by flies and removed from the alimentary tract of the insects within a short time after feeding can be injected into animals and produce the disease but it is still an unsolved question whether some one or more of the horse flies common to this locality may be a host for a generative stage of the parasite.

Jennings[2] believed at the beginning of an epizoötic that the infection might gain its foothold through the bite of a tabanid fly and that very likely only one species was involved. Since

the days of Darling and Jennings, we have learned that many species of the tabanid flies are common to the Isthmus and they breed and range through forested as well as unforested regions. Some circumstances connected with the history of the disease indicate that these flies are open to suspicion as vectors but proof will be hard to secure since these insects, in our experience cannot be kept in captivity for a period long enough to study the question. Studies are in progress with ticks, mosquitoes, flies and *Triatoma geniculata*, but up to the present there is nothing of note to report. The important local epizoötics that we have observed since 1909 have always been confined to the coastal plains or the swampy regions along lakes and rivers where several of the tabanid flies are known to breed.

Animals Susceptible to the Disease

A special report[7] by Clark and Dunn contains the details in regard to this subject but it may be stated briefly that all of our local wild and domestic animals as well as laboratory animals can be experimentally infected and they usually die in a few weeks. Animals infected that lived many months are as follows:

The small native mule (3), from 10 to 16 months.

The domestic cat, 1 year and 12 days.

The white tailed deer, *Odocoileus chiriquensis* Allen, 4 months.

The brocket deer, *Magama sartorii reperticia* Goldman, 6 months.

The collared peccary, *Pecari angulatus bangsi* Goldman, 10 months.

Animals infected but which recovered spontaneously are as follows:

Goat (half grown): Negative for the infection in a very few days.

Sheep (adult): Became negative in about 7 months.

Hog (48 lbs.—domestic): Became negative in about 2 months.

Calf (8 months old): Became negative to guinea pig tests in about 4 months.

Chickens could not be infected.

Iturbe[8] states that in South America spontaneous infections have been found in the capybara, the domestic dog, the fox and certain species of monkeys but this has not been the case with our species of capybara, the dog or our local species of monkeys.

The only animals, aside from the solipeds, that we have ever found carrying a spontaneous infection of *T. hippicum* were cattle. A herd of cattle ranging with a herd of horses and

mules that were attacked by the disease failed to show the presence of *T. hippicum* in blood-films but by inoculations of 2 to 4 cc of blood from the jugular vein into guinea pigs it developed that 4.5 per cent of the cattle were carrying the parasite without harmful results. Our calf experiment indicates that such cattle may be infective to guinea pigs for four months, thus the dangerous bat reservoirs, next in importance to the solipeds, are cattle. Where cattle and horses range together without protection at night from vampire bats, the question of control of this form of trypanosomiasis becomes a serious problem. We think it safe to assume that the animal reservoirs of greatest importance for the perpetuation of *T. hippicum* in our locality are, in the order named, the mule, horse and cattle. Those of secondary importance are the wild and domestic hogs, deer, sheep, goat and domestic cat.

Immunity

Infected guinea pigs give birth to negative offspring and these offspring can be given the disease artificially and killed by it in the usual period of time. During the past 18 months, it has been possible to follow the dams and foals in eight instances and the colts were all negative even though six of the dams were positive. Including colts up to one year of age and their dams, it is possible to list 27 with the following results noted during the observation period of the epidemic:

Dams positive and colts negative	14
Dams negative and colts positive	9
Dams and colts, both positive	4

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

Variation in Strains of T. Hippicum

Guinea pigs inoculated from different horses in the same epizoötic do not always behave the same. Some guinea pigs will live many months while others live but a few weeks. When such strains are passed from pig to pig for several months, the average life of the pigs is found to be about the same. One of our strains (colt 22) has killed 33 pigs in an average time of 31 days, yet the range was less than a month up to five months. A second strain (mare 31) has killed 36 pigs in an average time of 37 days and with a range of less than a month up to five months.

Local Records of the Disease

The first established record of a case was of course made by Darling,[1] in 1909, but the clinical character of the disease and losses due to it seem to have been known long before this date. The records passed down through one old family that has always been engaged here in stock-raising indicates that epizoötics of this character did not occur in Panama until large business contacts with other nations started the importation of stock. Whatever may be the facts regarding introductions from outside, it is certain that the disease has been enzoötic in the southern half of Panama for a very long period of time. Outbreaks of the disease have not appeared in Panama during the last 22 years with seasonal regularity, yet most of the widespread epizoötics are said by local stock-owners to have made their appearance in the months from September to January. There is usually a period of a few years between large epizoötics. We believe this may be due in part to the slow replacement of animals killed by an epizoötic, thus greatly reducing the number of contacts or exposures. Epizoötics of major or minor degree, in our experience, have occurred in every month of the year.

Recent Regional Surveys

We found this disease in the stock-breeding farm of the Panama Canal at Miraflores, on December 29, 1929. This farm and the stables border the west side of Miraflores Lake. It is said that eight deaths had occurred in the herd from October to December, 1929. Immediate steps were taken to conduct a blood-film survey of all Canal Zone horses, mules and burros as well as available animals as far down the Pacific coast as Chepo and as far up the coast as Aguadulce and Las Tablas. An area on the Atlantic coast and another on the Pacific coast lying along the boundary of Costa Rica also were examined. (See table I.)

During the year 1930, the main focus of infection on the Pacific side was at Miraflores and it extended a few miles up the coast as far as Venado Beach and Bruja Point. A second focus was found on the Atlantic end at and below the Gatun Dam and along the Chagres River below the Spillway. This last named focus spread during 1931 along the west bank of Gatun Lake and perhaps caused in addition the four cases at Fort Davis on the east side of the dam. The Miraflores focus extended to the Chiva Chiva Trail area across the canal but did not invade the Army herds at Fort Clayton and Corozal Post. Two

cases were found (1931) in the center of the Canal Zone, at Summit, which is also on the east side of the canal. At the close of the year 1931, and during the first half of 1932, a focus appeared about Pacora and Chepo.

The Panama Canal placed a quarantine on the Miraflores and Gatun Dam foci. The herd at Miraflores Stock Farm was kept in quarantine pastures and was subjected to treatment after a few of the old and useless members of the herd had been destroyed. This disease always has been considered fatal and it has been the custom since 1910 either to kill or isolate in screened quarters every case of the disease found in the Panama Canal Zone.

TABLE I—*Results of surveys, 1930 and 1931.*

REGION OR ORGANIZATION	1930		1931	
	EXAM.	POS.	EXAM.	POS.
United States Army, Panama Canal Dept.	1,840	1	1,876	4
Pacific third of Panama Canal Zone	241	64	109	3
Central third of Panama Canal Zone	94	1	207	2
Atlantic third of Panama Canal Zone	157	13	65	1
West bank Gatun Lake	231	9	343	26
East bank Gatun Lake	7	0	67	0
Panama City—Sabans to Chepo, R. de P.	657	0	167	0
Colon-Cristobal	176	0	0	0
Paja to Aguadulce & Las Tablas, R. de P.	251	1	419	0
Almirante, R. de P., United Fruit Co	29	0	0	0
Puerto Armuelles, R. de P., U. F. Co	157	0	858	0
Totals	3,840	89	4,111	36

All herds were slowly decimated wherever the disease made its appearance. New trypanocidal drugs have been manufactured during the last ten or fifteen years for the treatment of human and animal forms of trypanosomiasis but no extended observations had been made on the use of these drugs against *T. hippicum*. The Miraflores herd was sufficiently valuable to warrant an effort to salvage the sick animals and to attempt to control the spread of the disease in the quarantined animals by the use of curative and prophylactic treatments. All animals found positive by repeated blood-film surveys in the other areas mentioned were destroyed, except in two areas to be discussed later. All past local experience with preparations of mercury, arsenic and antimony had not been satisfactory. Tartar emetic seemed to be the only drug that would kill *T. hippicum* but unfortunately an effective course of treatment with this drug was

extremely toxic and usually proved fatal. At the time this epizoötic first was recognized, none of the new trypanocidal remedies were available on the Isthmus in sufficient quantity for the treatment of the herd, so we collected the local supply of tryparsamide and Bayer 205 (naganol) and started treatment in this herd of brood mares, foals, juveniles, stallions, mules and burros. Later a large supply of Bayer 205 was secured. This non-metalliferous remedy was given with tartar emetic during most of the treatment period. The following is a brief account of the treatment administered by Dr. T. L. Casserly:

> The entire herd was first treated on January 9, 1930, using Bayer 205 intravenously. The drug was dissolved in distilled water and the dosage used was 4 grams for mature animals, 2 grams for colts and small animals.
>
> The second treatment was given on January 16, 1930, using tryparsamide in 5-gram doses.
>
> The third treatment was given on January 23, 1930, employing Bayer 205, averaging 3 grams to mature animals and 1.5 grams to colts and small animals.
>
> From February 9, 1930, to April 1, 1930, several animals whose blood-films still showed trypanosomes as well as several that showed clinical symptoms in the presence of negative blood-films were given a series of treatments with Bayer 205 plus 7 grains of tartar emetic. On April 1, 1930, another series of intravenous treatments was administered, using Bayer 205 and tartar emetic. (Three hundred grams of Bayer 205 and 800 grains of tartar emetic were given to 144 animals).
>
> Ten animals were treated again on April 8, 1930, and 111 treated on April 14, 1930, while 106 received another course on April 22, 1930. Due to induration and neck abscesses at the site of former intravenous treatments, 33 animals were not treated on April 14, and 28 were not treated on April 22 for the same reason. Some of these animals died or were killed but the rest were given treatments after the lesions of the neck had subsided. Following the treatment thus described, a few new cases and several relapses developed and general treatment again was given the herd by Dr. C. C. Clay, on October 23, 1930, October 31, 1930, and November 14, 1930, but tartar emetic was used with Bayer 205 only on animals positive for the disease, while Bayer 205 alone was given for prophylactic reasons to the animals with negative blood-films. Now and then an animal or two has had treatments given as late in our period of observation as August, 1931.

It is extremely difficult, even in expert hands, to treat a large herd of animals intravenously without allowing a little of the treatment to escape from the puncture in the vein after the needle has been withdrawn. Any escape of tartar emetic produces very unsatisfactory local results and not infrequently mural thrombi develop in the jugular vein. Sometimes only a patch of slightly indurated subcutaneous tissue occurred and that soon subsided. Many times large abscesses formed with an asso-

ciated jugular thrombosis and occasionally there was a fatal hemorrhage from the vein. This discouraged the curative as well as the prophylactic application of the drug, yet tartar emetic still seems to be the most effective trypanocide.

The greater part of the discouraging results of intravenous treatment with tartar emetic occurred in the young and unmanageable animals. (See table II.)

TABLE II—*Neck injuries following intravenous injections.*

AGE	ANIMALS TREATED	NECK INJURIES	
		NUMBER	%
Under 12 months	25	13	52.0
1 year to 4 years	66	17	25.7
5 years to 20 years	67	6	8.9
Totals	158	36	22.7

Twelve of these cases revealed only a patch of induration or non-discharging abscess. Sixteen animals had discharging abscesses. Seven animals had large open ulcers, jugular thrombosis and severe hemorrhage from the vein. One animal had a most extensive phlegmonous lesion of both sides of the neck and almost complete obstructive thrombosis of both jugular veins.

Recovery is apparently complete in 25 of these animals, but these lesions played an important primary or contributory rôle in the cause of death of eleven animals. Twenty-one of these accidents were in animals only under treatment for prophylactic reasons.

Table III shows that 51 cases were discovered by the first thick-blood-film survey and that in the 32 months which have followed there were 16 new cases and 16 relapses or the recurrence of parasites in the blood-films discovered. At the close of September, 1932, there were 34 cases of treated trypanosomiasis still in the Miraflores herd and apparently they are in good condition except for some that were killed for experimental reasons. Twenty-one (31.3 per cent) of the 67 cases never were found positive for trypanosomes after a full course of treatment had been given.

The cattle that had ranged with this herd were segregated in pastures several miles distant in November, 1931. It is interesting to note that no new cases have developed since then. This

offers supportive evidence that *cattle carriers* were infecting some of the vampire bats of that region.

Relapse occurred in 23.2 per cent of the horses and in 25 per cent of the mules. A gelding, three years old, under prophylactic treatment from January, 1930, to August, 1931, became positive for trypanosomes at the end of that period.

There have been 108 animals added to this original herd of 158 from January, 1930, to September 30, 1932. Sixteen of them

TABLE III—*Trypanosomes in blood-film survey by months (Miraflores Stock Farm herd).*

MONTH	HORSES	MULES	BURROS	TOTAL NEW CASES
1930				
January	44	7	0	51
February	0	0	0	0
March	3 n 5 r	0	0	3
April	4 n 6 r	0 1 r	0	4
May	0	0	0	0
June	0	0	0	0
July	0 1 r	0	0	0
August	0	0 1 r	0	0
September	2 n	0	0	2
October	2 n	1 n	0	3
November	0	0	0	0
December	0	0	0	0
1931				
January	0 1 r	0	0	0
February	0	0	0	0
March	0	0	0	0
April	0	0	0	0
May	0	0	0	0
June	0	0	0	0
July	0	0	0	0
August	1 n	0	0	1
September	2 n 1 r	0	0	2
October	1 n	0	0	1
November	0	0	0	0
December	0	0	0	0
1932				
January	0	0	0	0
February	0	0	0	0
March	0	0	0	0
April	0	0	0	0
May	0	0	0	0
June	0	0	0	0
July	0	0	0	0
August	0	0	0	0
September	0	0	0	0
Totals	59	8	0	67

n = new case.
r = relapse or recurrence of parasites.

were colts that are offsprings of the herd and all were negative and have remained negative to blood-film surveys. There were six colts, eight mule colts and two burro colts. The old animals added were 61 horses and 31 mules. Since September, 1931, an

TABLE IV—*General incidence of trypanosomiasis in Miraflores herd (thick-blood-film survey).*

ANIMALS	NUMBER EXAMINED	POSITIVE	
		NUMBER	%
Horses	138	56	40.5
Mules	16	8	50.0
Burros	4	0	0.0
Totals	158	64	40.5

TABLE V—*Deaths from all causes in Miraflores herd (January, 1930, to August, 1931).*

CAUSE OF DEATH	ANIMALS
Trypanosomiasis, uncomplicated	17
Trypanosomiasis with treatment sequelae or some form of external violence	15
Prophylactic treatment sequelae	8
Other causes unassociated with trypanosomiasis, old animals, unserviceable, etc	17
Total	57

old member of the herd relapsed and one old member became positive for the first time on September 24, 1931. Two of the new horses added to the herd became positive so soon after their arrival that it is difficult to say where they acquired the disease. The others were negative and have not contracted the disease by contact in pastures with the old herd.

DIAGNOSTIC METHODS USED ON MIRAFLORES HERD

We depended on monthly thick-blood-film surveys for the herd in general and on daily films for a week or ten days on clinical suspects. In addition, a guinea pig was inoculated by the intraperitoneal route with 2 to 4 cc of blood, using one pig to each horse. On only one occasion have we had a guinea pig develop the disease from equines in which we failed to find the parasite in the blood-film. At the close of a year of observation, we collected serum from the application of the complement-fixation

test (January 27, 1931) on 123 members of the Miraflores Stock Farm herd. Each specimen was carbolized 0.5 per cent and forwarded in refrigeration to the Bureau of Animal Industry, Washington, D. C. These specimens were divided on their arrival so that Major R. A. Kelser, V. C., U. S. A., Chief, Veterinary Laboratory Section, Army Medical Center, Washington, D. C., also could examine them. A number of these specimens were anticomplementary or otherwise unsatisfactory. Specimens were again collected on March 13, 1931, and sent to the same laboratories. These animals represent the survivors of the herd that had been under treatment for a year. Both laboratories made three separate tests of each serum, using *T. equiperdum* antigens that were about two weeks old. The second shipment of specimens were set up against freshly prepared antigens of *T. hippicum* as well as *T. equiperdum*. These antigens, when of the same age and density, can be expected to give the same results. A comparison of the results obtained from the combined records of monthly blood-film surveys and the complement-fixation test is given in table VI.

It must be stated that since these tests were performed, horse 17 became positive for trypanosomes (August, 1931).

In order to eliminate the confusion caused by the application of the complement-fixation test to a herd that had been under treatment for a long time, we checked these methods on another herd in the early stage of the disease and which had not been given treatment. This Escobal Farm herd (Standard Fruit and Steamship Company) was located on the west bank of Gatun Lake and was negative to a blood-film survey in August, 1930, but by May, 1931, the disease had appeared there. We repeated the blood-film surveys and also collected specimens of serum from 108 animals for the same laboratories to apply the complement-fixation tests. The results are given in table VII.

Note that all but two of this herd were mules. The Bureau of Animal Industry used a one-day-old antigen of *T. equiperdum* and a six-day-old antigen of *T. hippicum*, without getting important variations in the results of the test.

The Army Medical Center used *T. equiperdum* and *T. hippicum* antigens, each three days old, without appreciable variations in the result.

Both laboratories secured a positive diagnosis in mule 93. This animal showed ample clinical evidence of the late stage of the disease but numerous blood-films were examined and found negative for trypanosomes. On the other hand, mule 90 was

TABLE VI—*Comparison of results obtained from the combined records of monthly blood-film surveys and complement-fixation tests.*

LAB. No.	ANIMAL	TRYPANOSOMES IN THICK BLOOD-FILM	COMPLEMENT-FIXATION TEST	
			B. A. I.	ARMY M. C.
6	Horse	−	−	−
7	Horse	−	−	−
8	Horse	−	−	−
9	Horse	−	−	−
10	Horse	−	−	−
11	Horse	−	−	−
12	Horse	−	−	−
14	Horse	−	−	−
15	Horse	−	−	−
16	Horse	−	−	−
17	Horse	+	−	−
17X	Horse	−	−	−
21	Horse	−	−	−
22	Horse	−	−	−
30	Horse	+	++++	++++
32	Horse	+	−	−
35	Horse	+	−	−
37	Horse	+	−	−
38	Horse	+	−	−
39	Horse	−	−	−
41	Horse	+	−	−
42	Horse	+	−	−
44	Horse	+	−	−
46	Horse	+	−	++++
47	Horse	+	−	−
48	Horse	+	−	−
49	Horse	+	−	−
51	Horse	+	V. S. R.*	+−
52	Horse	+	−	−
53	Horse	+	V. S. R.*	+−
54	Horse	+	−	−
55	Horse	+	−	−
56	Horse	+	−	−
58	Horse	+	−	−
59	Horse	−	−	−
60	Horse	+	−	−
61	Horse	+	−	−
62	Horse	−	−	−
63	Horse	−	−	−
64	Horse	−	−	−
65	Horse	+	−	−
66	Horse	−	−	−
68	Horse	−	−	−
69	Horse	−	−	−
70	Horse	+	−	−
71	Horse	+	−	−
76	Horse	−	−	−
77	Horse	−	−	−
78	Horse	−	−	−
80	Horse	−	−	−
81	Horse	−	−	−
82	Horse	+	++++	++++

*Very slight reaction.

TABLE VI—*Comparison of results obtained from the combined records of monthly blood-film surveys and complement-fixation tests—Continued.*

LAB. NO.	ANIMAL	TRYPANOSOMES IN THICK BLOOD-FILM	COMPLEMENT-FIXATION TEST	
			B. A. I.	ARMY M. C.
83	Horse	−	−	−
84	Horse	−	−	−
85	Horse	+	−	−
86	Horse	−	−	−
87	Horse	−	−	−
88	Horse	+	++++	++++
89	Horse	−	−	−
90	Horse	+	−	−
92	Horse	−	−	−
94	Horse	+	−	−
95	Horse	−	−	−
96	Horse	−	−	−
97	Horse	g. p. +	−	−
98	Horse	−	−	−
99	Horse	−	−	−
101	Horse	−	−	−
102	Horse	−	−	−
103	Horse	−	−	−
106	Horse	−	−	−
109	Horse	−	−	−
110	Horse	−	−	−
112	Horse	−	−	−
113	Horse	−	−	−
121	Horse	−	−	−
123	Horse	−	−	−
124	Horse	−	−	−
125	Horse	−	−	−
126	Horse	−	−	−
127	Horse	−	−	−
129	Horse	−	−	−
131	Horse	−	−	−
132	Horse	−	−	−
133	Horse	−	V. S. R.*	−
134	Horse	−	−	−
136	Horse	−	−	−
138	Horse	−	−	−
139	Horse	−	++++	++++
140	Horse	−	−	−
141	Horse	−	−	−
142	Horse	−	−	−
145	Horse	−	−	−
146	Horse	+	−	−
156	Horse	−	−	++++
160	Horse	−	−	−
161	Horse	−	−	−
162	Horse	−	++++	++++
163	Horse	−	++++	++++
169	Horse	−	−	−
171	Horse	−	−	−
172	Horse	+	−	−
174	Horse	−	−	−
176	Horse	−	−	−

*Very slight reaction.

TABLE VI—*Comparison of results obtained from the combined records of monthly blood-film surveys and complement-fixation tests—Concluded.*

LAB. NO.	ANIMAL	TRYPANOSOMES IN THICK BLOOD-FILM	COMPLEMENT-FIXATION TEST	
			B. A. I.	ARMY M. C.
177	Horse	−	−	−
178	Horse	−	−	−
25	Mule	+	−	−
72	Burro	−	−	−
73	Burro	−	−	−
74	Burro	−	−	−
114	Mule	+	−	−
116	Mule	−	−	−
118	Mule	−	−	−
122	Mule	+	−	No test
143	Mule	−	−	−
151	Mule	−	−	−
159	Burro	−	−	+−
167	Mule	−	−	−
168	Mule	−	−	−
170	Mule	−	−	−
173	Mule	−	−	−
179	Mule	−	−	−
181	Mule	−	+	−

diagnosed easily by the use of the blood-film and was negative to the complement-fixation tests of both laboratories.

Both the blood-film survey and the complement-fixation test should be used for diagnostic purposes, and in special cases a guinea-pig inoculation should be added. However, it seldom happens that a few frequently repeated thick-blood-film examinations fail to establish a diagnosis in our local form of the disease. It is not an easy nor an inexpensive matter to keep a fresh satisfactory antigen for daily use, yet in the late stage of a chronic or treated case one must have recourse to this diagnostic method. In order to learn how early the complement-fixation test would definitely respond to the disease we inoculated a normal horse and mule with our local strain of *T. hippicum*. On May 25, 1931, these animals were given intravenous injections of guinea-pig blood that contained an abundance of the parasites. The mule was given 2 cc and the horse 2.5 cc of this blood and both animals were positive for trypanosomes the next day. The mule had eight parasites in its thick-blood film and the horse ten parasites.

Subcutaneous inoculations usually require five to seven days before parasites are found in the blood-films. These animals were allowed to run an untreated course for several weeks. Blood

TABLE VII—*Comparison of results obtained from the combined records of monthly blood-film surveys and complement-fixation tests (recheck).*

LAB. NO.	ANIMAL	TRYPANASOMES IN THICK BLOOD-FILM	COMPLEMENT-FIXATION TEST	
			B. A. I.	Army M. C.
1	Mule	−	−	−
2	Mule	−	−	−
3	Mule	−	−	−
4	Mule	−	−	−
5	Mule	+	++++	++++
6	Mule	−	−	−
7	Mule	−	−	−
8	Mule	−	−	−
9	Mule	+	++++	++++
10	Mule	−	−	−
11	Mule	−	−	−
12	Mule	−	−	−
13	Mule	−	−	−
14	Mule	−	−	ac.*
15	Mule	−	−	−
16	Mule	−	−	−
17	Mule	−	−	−
18	Mule	−	−	−
19	Mule	−	−	−
20	Mule	−	−	−
21	Mule	−	−	−
22	Mule	−	+	−
23	Mule	−	−	−
24	Mule	−	−	−
25	Mule	−	−	−
26	Mule	−	++	+
27	Mule	−	−	−
28	Mule	−	−	−
29	Mule	−	−	−
30	Mule	−	−	−
31	Mule	−	−	−
32	Mule	−	−	−
33	Mule	−	−	−
34	Mule	−	−	−
35	Mule	−	−	−
36	Mule	+	++++	++++
37	Mule	−	−	ac.*
38	Mule	−	−	−
39	Mule	−	−	−
40	Mule	−	−	−
41	Mule	−	−	−
42	Mule	−	−	−
43	Mule	−	−	−
44	Mule	−	−	−
45	Mule	−	−	−
46	Mule	−	−	−
47	Mule	−	−	−
48	Mule	−	−	−
49	Mule	−	−	−
50	Mule	−	−	−
51	Mule	−	−	−
52	Mule	−	−	−
53	Horse (gelding)	−	−	−
54	Mule	−	+	++

*Anticomplementary.

TABLE VII—*Comparison of results obtained from the combined records of monthly blood-film surveys and complement-fixation tests (recheck)—Concluded.*

LAB. NO.	ANIMAL	TRYPANASOMES IN THICK BLOOD-FILM	COMPLEMENT-FIXATION TEST	
			B. A. I.	Army M. C.
55	Mule	−	−	−
56	Mule	−	−	−
57	Mule	−	−	−
58	Mule	−	−	−
59	Mule	−	−	−
60	Mule	−	−	−
61	Mule	−	−	−
62	Mule	−	−	−
63	Mule	−	−	−
64	Mule	−	−	−
65	Mule	−	−	−
66	Mule	−	No record	−
67	Mule	−	−	−
68	Mule	−	−	++++
69	Mule	−	−	−
70	Mule	−	−	−
71	Mule	−	No test	−
72	Mule	−	−	−
73	Mule	−	−	−
74	Mule	−	−	−
75	Mule	−	−	−
76	Mule	−	−	−
77	Mule	−	−	−
78	Mule	−	−	sc.s
79	Mule	−	−	−
80	Mule	−	−	−
81	Mule	−	−	−
82	Mule	−	−	−
83	Mule	−	−	−
84	Mule	−	−	−
85	Mule	−	−	−
86	Mule	−	−	−
87	Mule	+	++++	++++
88	Mule	+	++++	++++
89	Mule	+	++++	++++
90	Mule	+	−	−
91	Mule	+	++++	++++
92	Mule	+	++++	++++
93	Mule	−	++++	++++
94	Mule	+	No test	+++
95	Mule	+	++++	++++
96	Mule	+	++++	++++
97	Mule	+	++++	++++
98	Mule	−	+	−
99	Mule	+	++++	++++
100	Mule	−	−	−
101	Horse (gelding)	−	++++ (Control sl.ac.*)	++++
102	Mule	−	−	−
103	Mule	+	++++	++++
104	Mule	+	No specimen	No specimen
105	Mule	+	No specimen	No specimen
106	Mule	−	No specimen	No specimen
107	Mule	−	No specimen	No specimen
108	Mule	−	No specimen	No specimen

*Anticomplementary.

specimens were collected for the application of the complement-fixation test, 16, 24, 31 and 38 days after the two animals had been inoculated. These were sent to the same laboratories for examination and each specimen was reported as positive for the disease. We had not anticipated so early a response to the test or earlier specimens would have been forwarded. Both animals revealed a very definite clinical picture by the time the third specimen was forwarded. The horse, alone, showed marked edema of the belly. These animals were now placed on treatment using tryparsamide. Five human doses (10 grams) were taken as one dose for an animal. This treatment has been continued every five to seven days during a period of six weeks. Their physical condition has improved but daily blood-films have been positive in almost every instance. The parasites are present in a sparse manner as a rule, but about once a week they are found in great numbers.

On August 13, 1931, the mule had over 200 parasites in one thick film while the horse, on August 17, 1931, had over 800 parasites in its film. Treatment was started on the horse on June 29, 1931, and on the mule on July 6, 1931. The dose of the drug was increased to 12 grams. The daily blood-film continued to be positive for trypanosomes up to September 22, 1931, when the treatment was changed to a series of three doses of Bayer 205 plus tartar emetic. The mule showed a few trypanosomes for the next two days after the first dose, but both the horse and mule remained steadily negative from that date forward. The mule, at the time this is written (October, 1932), is in good physical condition and is performing the duty of a saddle animal for the Cattle Industry. The horse later was used in an experiment to see whether the disease and its treatment developed any degree of immunity against a new infection by the same parasite. It was given 2 cc of blood from the heart of guinea pig 51 that was positive for *T. hippicum*. This was an intrajugular injection, made January 24, 1932. It again acquired the infection but never revealed many trypanosomes in its blood-films. The clinical picture of the disease reappeared and its condition became so helpless that on March 11, 1932, we killed it.

Associated Diseases Found

The general application of the thick-blood film during the first 19 months of observation of the Miraflores herd shows that 27.8 per cent had piroplasmosis and 20.2 per cent had filariasis. It is safe to conclude that every animal raised on the ranges in this

region becomes a carrier of these diseases as soon as it becomes a few months old. The only acute cases of piroplasmosis that we find are those which occur in recently imported horses used as mounts for the Army and in young colts and foals in the rural herds. Almost every autopsy examination reveals several adult filaria in the peritoneal cavity.

Spirochetosis: The general surveys of the herds in the Republic of Panama and the Canal Zone revealed four animals positive for this disease. Three of them were very scant infections, while one was very abundant. We do not believe that the infection is very important from the viewpoint of the clinician.

Laminitis: This was found in its usual incidence among old animals and the lesion seems to be provoked or intensified by the use of trypanocidal drugs. Indeed, some cases of laminitis appear to be directly caused by the drug. These drugs also caused many animals to be stiff and lame for a day or two after treatment. Many animals also developed a dry painful swelling of the anal and vulvar tissues, with radial fissures that oozed and required attention to prevent myiasis. Two animals did develop anal myiasis (*Cochliomyia macellaria* Fab.). Two abortions occurred following treatment but the disease may have been as much responsible for these accidents as the drug.

Intestinal parasites: These are, of course, very common and many extremely heavy infestations were found in animals from six months to two years of age. Strongylidosis, as a severe infection, has been present in every horse and mule over a few months of age that the senior author has examined at autopsy during the past three years in various parts of Panama and the Canal Zone. Every autopsy has revealed either a verminous aneurysm of the mesenteric artery or chronic obliterative arteritis of this vessel with marked stenosis. We are inclined to attach more importance to this arterial lesion than is usually given it by writers. One must admit at once that a great many animals manage to lead a long life with perhaps few serious periods of interference from this disease. On the other hand, many serious complications do occur and these are probably more common in young adult animals. Serious accidents are just as apt to be associated with small aneurysms as large ones. These lesions of the mesenteric artery are very easy to overlook at autopsy because they usually form a mass about the size and shape of a lemon that is buried in the mesenteric tissues and surrounded by a cluster of enlarged lymph-nodes. The aneurysms

are located usually at a distance of a few centimeters from the wall of the aorta.

We do not feel that "colic" is the most frequent clinical manifestation of the disorder. Unexplained emaciation, anemia and weakness in young animals seem to be the best guide to a clinical diagnosis. A vast number of these aneurysmal sacs, in animals acutely ill as a result of this disease, are filled with either organized or recently formed thrombi as well as the larvae of the parasites. The sac, when emptied, very frequently shows one or more necrotic spots in some part of its wall that involves all coats of the vessel. These necrotic areas are usually well supported against rupture by the acute and chronic inflammatory processes set up in the tissues immediately outside the sac. This disease of the mesenteric artery is a great handicap to a herd when it is invaded by other diseases and particularly by trypanosomiasis. The latter disease is accompanied by severe anemia as well as by the increased danger from thrombosis due to the toxic effects of trypanocidal drugs.

We have seen no evidence at autopsy that the three trypanocidal drugs we employed were effective against the larvae in the aneurysmal sacs. We feel reasonably certain that nine deaths in the Miraflores herd can be ascribed chiefly to accidents in verminous aneurysms of the mesenteric artery, such as embolism and thrombosis and some obstruction to the arterial blood supply which must in turn have interfered with the assimilation of food. An effective treatment directed at the larval stage of these worms might also enhance the danger of embolism since the dead larvae may become foreign bodies in the blood-stream. We have accepted a number of opportunities to examine animals killed because they were unserviceable, that is, no definite reason could be assigned for their condition, such as the presence of a well-defined disease or injury or advanced age. All of these animals revealed either stenosis or dilatation of the mesenteric artery of a severe degree due to strongylidosis. There was marked interference with the function of this arterial tree.

Many young animals in the Miraflores herd were treated for worms by the oral administration of tartar emetic and in addition they received routine treatment intravenously with Bayer 205 plus tartar emetic for trypanosomiasis. We sacrificed some of these animals to note the result of treatment and were disappointed when we found living larvae in the aneurysmal sacs and also many living parasites fast to the mucosa of the cecum. It is also worthy of note that living adult filarial parasites were

found in the peritoneal cavity and that in some instances piroplasms also could be detected in blood-films following treatment.

Prophylactic Treatment

The many evil results following such treatment in the Miraflores herd and the need of very frequent treatments actually to prevent infection have caused us to decide that such management of a herd in an epizoötic is not warranted. Furthermore, the expense involved would soon be out of all proportion to the market value of the animals.

Segregation

A diligent laboratory service soon can weed out the positive animals so that segregation and treatment can remove the risk of the spread of the disease from such animals. Protection of the negative animals can be secured by stabling them in illuminated or screened barns. The infected bats will live only about a month and the only "carrier risks" left at large are the cattle and other animals already pointed out that become light carriers of the parasites for a few months. We have known for many years that animals kept continuously in stables seldom develop trypanosomiasis. They are far more apt to be free from the attacks of bats, horse-flies and other insects, as well as exposed to a much less extent to the mechanical means of transmission. Even pasture segregation in a wide agricultural region can be of considerable benefit. The Miraflores herd was found divided in four pastures relatively close together around the same arm of Miraflores Lake. All of these pastures developed some cases of trypanosomiasis, but a very large majority of the cases came from one pasture. Segregation is an easy matter with local Army animals for they are kept in stables lighted by electricity and are never on pasture. Even during maneuvers through the jungle and the interior these animals are kept on a picket line and fed, and to some extent protected by lanterns. They avoid as far as possible any contact with range or village animals. Animals kept in this manner are open to daily inspection and a sick horse or mule is found in an early stage of its illness and placed in a quarantined stall. These animals are well groomed, scores are under daily treatment and ticks removed and their bites treated. There are three collections of Army animals on the east bank of the Panama Canal directly opposite two foci of the disease on the west bank of the canal. One period of Army maneuvers has occurred during the epizoötic. Notwith-

standing these conditions, we have had but five cases of the disease in almost 2,000 Army animals.

Strict quarantine of the diseased areas, of course, was of some assistance. We were not so sure what results might be expected in rural animals that were kept part of the time in stables and part of the time in pastures. An owner is in great difficulty when this disease is present in a herd, since he cannot safely add replacement animals during an epizoötic and he dislikes sacrificing animals that the microscopic examination alone shows are positive and in which there is no sign of illness to be found on inspection or by the use of the animal. He receives no pay for the animals killed under these circumstances.

The Escobal Farm of the Standard Fruit and Steamship Company had a herd of this character located on the west bank of Gatun Lake about 45 minutes launch ride from Gatun, the nearest Canal Zone village. We performed ten thick-blood-film surveys at intervals of about three days and applied one complement-fixation test to the herd. The positive cases were collected as they were discovered in one small banana farm about four miles from the clean members of the herd. There was a ridge of about 400 feet elevation between the clean and infected animals. All late-stage cases of the disease were killed, but the other positives were worked until they became unserviceable before they were sacrificed. Some of these animals were of service for two or three months. Three of them, small mules, are still living at the end of 16 months, but are in very poor condition. No new cases have appeared in the clean members of the herd nor in the new animals added to the main herd. It is of interest to note that almost no cattle ranged the area in which this herd was used.

A second herd located not far away contained a smaller collection of animals. We have adopted the same method of management except that all positive cases were killed and this area also has remained free of the disease. It would appear from these experiences that mechanical means of transmission should be given due consideration as well as the natural vector. The perpetuation of the disease in a region is no doubt due to such a vector and some isolated animal reservoirs. The senior author has placed a guinea pig with clipped ears in a closed pen with one infected guinea pig with a clipped bleeding ear and managed to infect the clean pig. This cannot happen with much success unless straw or grass is placed on top of them in crowded quarters. This forces them to run in tunnels through such grass

or straw and they pick up wet fresh blood on the freshly clipped ear.

During the construction period of the Panama Canal we were inclined to believe that this disease would be eradicated in the Canal Zone within a few years. We based this belief on the hope that the Canal Zone would remain a depopulated region for domestic animals as well as man and that transportation would be motorized to such a large extent that the disease could not perpetuate itself in the small number of scattered stabled animals required by the Panama Canal and the United States Army. The period of operation and maintenance of the Panama Canal has now been in effect many years and equine trypanosomiasis is still present and reaches at times epizoötic proportions.

The idea of a depopulated Canal Zone was abandoned. Farms, large and small, are scattered all over the Canal Zone and herds of animals are found in villages along the boundary. Trial systems connect certain parts of the Canal Zone with these contact villages. It is more or less easy to control the disease in animals under the direction of organizations like the Army and the Panama Canal and large agricultural organizations but it is difficult to follow the condition of each small private owner's stock. Such animals are potential reservoirs that can remain concealed for a long time. The Panama Canal, the Army and large agricultural organizations have learned from experience that they cannot motorize to the extent they planned a few years ago. It is certain that a relatively large number of horses and mules will always be required. The difficulty in maintaining control of the disease is greater at present than in the past because of the road-building which permits easy and inexpensive transportation of cheap work animals, a class that formerly was not worth ocean transportation.

Trypanosomiasis has seldom gained a foothold on the west side of the Canal Zone. The new road system now open for use through the upper provinces of Panama already has permitted the disease to become established about 200 miles beyond the west side of the Canal Zone. These roads soon will 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 to the United States unless vigorous steps are taken to check its advance. The senior author has had opportunities to survey large herds of stock in the Atlantic coastal plains of Costa Rica, Honduras and Guatemala, but did not find a case of the disease in these regions. It was found on the Atlantic coast of Colombia.

A closely related equine trypanosomiasis, dourine,[5] in the past has appeared in nine of the middle west and southwestern states as well as Canada and the time and expense involved in its control should focus serious attention on the possibility of this form being introduced.

There are many animals, particularly mules, afflicted with an early stage of the disease that could travel the road system from Panama to Texas before the disease would plainly manifest itself to a casual examination by an inspector. Diagnosis depends chiefly on laboratory methods in the early stage of the disease.

SUMMARY

1. The first scientific record made in regard to the presence of equine trypanosomiasis (murrina or derrengadera) on the isthmus of Panama was written by Darling, in 1910. He establblished the fact that it was a trypanosomiasis and he named the pathogenic agent *Trypanosoma hippicum*. The clinical character of the disease and losses due to it seem to have been known long before this date. The local belief is that epizoötics of this character did not occur in Panama until large business contacts with other nations started the importation of stock. Whatever the facts may be regarding introduction from outside, it is certain that the disease has been enzoötic for a very long period of time in that part of the republic which includes the Canal Zone and extends to the boundary of Colombia. Outbreaks of the disease have not appeared in Panama during the last 22 years with seasonal regularity, yet local stock-owners state that most of the widespread epizoötics have made their appearance in the months from September to January. There is usually a period of a few years between epizoötics. We believe this is due in part to the slow replacement of animals killed by an epizoötic, thus greatly reducing the number of contacts and exposures. New cases and epizoötics of major or minor degree, in our experience, have occurred in every month of the year. The disease appeared again in epizoötic manner in the Panama Canal Zone during the close of the year 1929. There were two foci on the west bank of the Canal Zone. One occurred at the Mariflores Stock Farm, near the Pacific terminus of the canal, while the other appeared at the Atlantic terminus, between the Gatun Dam and Cristobal. A focus also was found about 200 miles west of the Canal Zone in the republic of Panama. During the late months of 1931 and the early months of 1932, it ap-

peared about 30 miles down the Pacific coast from the city of Panama, in the Sabanas between Pacora and Chepo.

2. *Trypanosoma hippicum* and the disease it produces seems to us to correspond to that found in Colombia. Now that we have learned that *T. hippicum* can be carried by cattle, there is more supportive evidence that inclines us to share Wenyon's believe that *T. hippicum* and *T. venezuelense* are simply races of *T. evansi* and that they differ from it only in minor details.

3. The clinical picture of the disease is exhibited by fever of an irregular character, progressive emaciation, anemia, faint icterus, a rough coat and sometimes edema of the most dependent part of the abdominal wall and sheath and to a less extent of the legs. Late in the illness there is a marked weakness of the posterior extremities and the animal walks with a stiff, staggering gait, frequently dragging the foot. There is no impairment of appetite throughout the course of the disease. It is frequently possible to find the trypanosome in the blood-film of an animal two or three weeks before clinical evidence of the disease appears. For this season, as soon as a single case is recognized, a general laboratory survey of the herd should be made. Such a survey may consist of blood-film examinations for the detection of trypanosomes, inoculations of susceptible animals and the application of the complement-fixation test for equine trypanosomiasis. We select the thick-blood-film method for rapid field surveys, since it is difficult to examine fresh blood-films in the field in cloudy, rainy, windy weather. A herd should be examined every two or three days, for at least ten surveys, in order to pick out all of the cases that are active or are in the incubation period. When it is possible to have recourse to the complement-fixation test, this will be of great assistance but in most field work where we have daily access to a herd we prefer the blood-film survey, supported by guinea-pig inoculations in selected cases.

4. The recent work of Dunn[6] in his study of the local bats, has established beyond doubt that the vampire bat, *Desmodus rotundus murinus* Wagner, is an important natural vector of this disease and that it can disseminate the disease on its nightly feedings over an average period of about one month after it has acquired the disease and before death overtakes it as a result of the disease. It adds additional proof that this trypanosome can pass through the normal mucosa of the alimentary tract. The question of natural insect vectors (horse-flies) is still open. However, our epizoötics of this disease are found only in herds

where the disease has been given several months to spread and in the case of an insect vector the epizoötic should appear in an explosive manner. We believe that the vampire bat is probably the main vector that keeps the disease enzoötic. Mechanical means for the spread of the disease must be given due consideration, for we know that any means of transferring wet, infected blood to breaks in the skin of a negative animal or into the mucous tracts of an animal can convey the disease. Coition is probably as important a means of transfer as any other mechanical means.

5. We can experimentally produce this form of trypanosomiasis in all of the wild and domestic animals that we have been able to collect, with the exception of the chicken. Nearly all of them, however, die within a few weeks after they acquire the infection. The small, native mule, domestic cat, white-tailed deer, brocket deer and collared peccary (wild hog) can live from four to 16 months before death supervenes. In fact, we have three untreated mules that have survived 16 months and, although their physical condition is very poor, they will probably live several months longer. Animals that we have been able to infect but which recover spontaneously are the goat, sheep, domestic hog and a calf. *The only animals, aside from the equines, that we have found carrying a naturally acquired infection, were cattle.* Such stock ranging with an infected herd of horses and mules, when tested by guinea-pig inoculations, revealed the fact that 4.5 per cent *carried* this form of trypanosomiasis. Where equines and cattle range together day and night, without protection from vampire bats, the question of control of this form of trypanosomiasis becomes a serious problem to the owner. We think it safe to assume that *animal reservoirs* or *carriers* of great importance for the perpetuation of the disease in Panama are cattle and certain horses, mules and burros with a high individual resistance. Reservoirs of secondary importance are the wild and domestic hogs, deer, sheep, goat and the domestic cat.

6. Immunity is not conferred by infected guinea pigs to their offspring and the foals of infected mares can acquire the disease. One experiment horse, that was not treated until it was in a critical advanced stage of the disease, apparently was cured. Its physical condition was good and guinea pigs inoculated with blood from its jugular vein did not acquire the disease. We successfully reinfected this animal and it died of the disease,

although it never had the abundant number of parasites that had appeared in the initial infection.

7. The strains of *T. hippicum* do not always behave in the same manner on initial infections secured in guinea pigs from different horses and mules, but this may be due to individual peculiarities in the animals, since, when carried through a long series of guinea pigs, the average duration of the disease is about the same. The strain secured from *cattle carriers* of the disease ran a typical clinical course of the disease, ending in death in the average time expected. The trypanosome was similar in all respects to *T. hippicum* recovered from horses and mules in an epizoötic except that the majority of the parasites revealed either a very small centrosome or none at all. The introduction of cattle, horses and mules from all parts of the world and, in particular, from the north coast of South America should have succeeded in bringing in almost any form of trypanosomiasis that could survive the period of transportation by sea. This is another reason for considering the possibility of a very close relationship between all our tropical and subtropical forms of equine trypanosomiasis common to the western hemisphere.

8. No treatment was in local use for the disease until a few years ago. It was simply a question of surveying all animals and then quarantining or killing the positives found. A number of new trypanocidal drugs have appeared in the past fifteen years and the Miraflores epizoötic gave us a chance to try some of these. We were forced to choose those that happened to be in stock on the Isthmus and these were tryparsamide, naganol (Bayer 205 vet.) and tartar emetic. Tryparsamide did not kill the infection even over a long course of treatment in a horse and mule, but it seemed to offer some protection, since the animals ran a longer course than usual without getting off their feet. The use of naganol gave better results but was followed by too many relapses. Our past experience with tartar emetic proved to us that it was an effective trypanocide but the drug was too toxic for the host. We compromised by using a half-dose of tartar emetic with a full dose of naganol. There seems to be something in naganol that guards the action of tartar emetic. At any rate it is the first time that we have been able to treat any animal with the disease successfully. It is our practice at present to treat them as follows: Horses and mules over 600 lbs. in weight receive 8 grams of naganol and 21 grains of tartar emetic divided into three doses. The

first dose consists of 4 grams of naganol and 7 grains of tartar emetic; the second dose is 2 grams of naganol and 7 grains of tartar emetic and the third dose is the same as the second. Each dose is dissolved in 200 cc of distilled water and injected into the jugular vein by gravity. Six days intervene between these injections or doses. It is extremely difficult, even in expert hands, to treat a large herd of animals in this manner without the escape of some of the drugs from the punctured vein. Bad results follow the least escape of the drug. Sometimes only a patch of slightly indurated tissue occurred that quickly subsided. Several times large abscesses formed with an associated jugular thrombosis and occasionally a fatal hemorrhage from the jugular vein. Most of these bad results occurred in young, unmanageable animals. We have ceased using or advising the use of prophylactic treatment since it must be given too frequently, it is too expensive, it is followed by too many bad results and, furthermore, the control of the disease can be accomplished better by other means. The records show 51 cases in 158 animals belonging to the Miraflores herd, when control of the situation was undertaken. In the 32 months of observation that have followed (this includes 108 additions to the herd), 16 new cases have developed and 16 relapses have occurred. At the close of September, 1932, there were 34 of the positive cases looked upon as cured. Some of these were sacrificed for study, while many of them are on duty and seem to be in normal condition. Twenty-one of the positive cases were never found positive after the first full course of three treatments was given. No new cases have developed in the past eleven months. During this period of time no cattle have ranged with the herd. The original herd has received 108 additions since the first diagnostic surveys were made (from February, 1930, to September, 1932). Sixteen of them were colts that are the offspring of the herd, 61 horses and 31 mules. Since September, 1931, an old member of the herd relapsed, one old member became positive for the first time and two additions to the herd became positive so soon after their arrival that doubt exists as to whether they came infected or acquired infection later. Notwithstanding the wonderful advance made in the manufacture of trypanocidal drugs, it is still necessary to seek for something that can be more cheaply, easily and safely administered. The majority of the animals in this region are not valuable enough to warrant the expense of the prophylactic and curative courses as recommended by the manufacturers of these drugs. A safe drug that

can be purchased easily and administered by the average owner of cheap work animals is needed.

9. The general use of the blood-film surveys during the first 19 months of observation of the Miraflores herd showed that 27.8 per cent had chronic piroplasmosis and 20.2 per cent had filariasis. It is safe to conclude that every horse and mule that grows up on the local ranges acquire these infections. The only acute cases of piroplasmosis that we have found were those that occurred in animals recently imported from the United States or colts born on the local farms. Spirochetosis was found four times in our general surveys. We do not consider it very important from the clinical viewpoint. Intestinal parasites are, of course, very common and many heavy infections were found. Strongylidosis, as a severe condition, has been present in every animal above a few months of age. The verminous aneurysms of the mesenteric artery and mesenteric arteritis connected with this infection are serious factors. Many animals lead a long life with this condition present but it is a serious potential source of trouble. At any time the animal is brought below par, a mural thrombosis is apt to develop that partially or completely cuts off the lumen of this important vessel. It is of interest to note that the use of tartar emetic by mouth and by intrajugular injections have in most cases failed to kill larvae in these aneurysms as well as many that attached to the mucosa of the cecum. Osteomalacia (bighead) was recorded in five animals as well developed cases of this disease. Laminitis was found in its usual incidence in old animals. The lesion is intensified by the use of the trypanocidal drugs. Indeed, some cases of acute laminitis appear to be the direct result of the use of the drug.

10. Cardinal features in the management of a herd infected with this disease should consist of numerous laboratory surveys, frequently repeated, employing the blood-film, animal inoculations and the application of the complement-fixation test in order to segregate the positives for treatment and to destroy old or useless animals. Protect mules and horses in screened or illuminated stables from dusk to daylight to prevent bat biting. Avoid ranging horses and mules with cattle. Treat all open sores and cuts to avoid mechanical transfers of the disease from animal to animal and prevent coition until the herd is again clean.

11. Murrina or derrengadera presents a different problem in control measures than dourine. The latter is said to be transmitted almost solely by coition and measures for the suppression

of dourine are directed only at this method of the spread of the disease. Murrina also can be transmitted by coition, but mechanical means, the animal reservoirs and the vampire bat must be given attention in control measures. The disease is not limited to brood mares and stallions. Colts, mules and young geldings become victims of the disease and even cattle are found with natural infections.

12. We have never been able to establish a laboratory diagnosis of this disease west of the Canal Zone until January, 1930, when we found it as far up the Pacific coast as Aguadulce, a distance of about 200 miles. We consider the disease enzoötic in the Canal Zone and that part of the republic of Panama next to Colombia. The completion of the international highway will favor the introduction of this disease northward and conceivably as far north as the United States unless quarantine methods are placed in effect. The disease can become established in a herd several weeks and sometimes months before ordinary inspection of the herd will show a serious loss of animals and sufficiently advanced stages of the disorder to cause a suspicion of its real nature.

ACKNOWLEDGMENTS

The Gorgas Memorial Laboratory wishes to express its thanks to the Health Department and Supply Department of the Panama Canal, to the Panama Canal Department of the United States Army and to the Republic of Panama for the opportunity offered to study this disease. The Supply Department of the Panama Canal, through J. H. K. Humphrey, Assistant Chief Quartermaster, has given us excellent opportunities for a long period of observation of the herd at the Miraflores Stock Farm and he is continuing with us a critical study of the disease. Thanks are due also Dr. C. C. Clay for his assistance in continuing treatment during the absence of Dr. T. L. Casserly. We are deeply indebted also to the Pathological Division of the Bureau of Animal Industry at Washington, D. C., and to the Veterinary Laboratory Section of the Army Medical School, Army Medical Center at Washington, D. C., for their kindness in building fresh antigens and setting up the complement-fixation tests. Our thanks are due the United Fruit Company and Standard Fruit and Steamship Company for their coöperation in the study of their herds.

REFERENCES

[1]Darling, S. T.: Equine trypanosomiasis in the Canal Zone. Bul. Soc. Path. Exot., iii (1910), 6.

[2]Darling, S. T.: Murrina. A trypanosomal disease of equines in Panama. Proc. Med. Asso. Isthmian Canal Zone, iii (1911), part 2.

[3]Wenyon, C. W.: Protozoology, i, p. 572. (William Wood & Co., New York, 1926).

[4]Barber, M. A., and Komp, W. H. W.: Some modifications of the thick-film method in the examination of blood for malaria parasites. International Conference on Health Problems in Tropical America, p. 110. (Published by the United Fruit Company, Boston, 1924.)

[5]Mohler, John R.: Dourine of horses. Farmer's Bul. 1146, p. 9.

[6]Dunn, Lawrence H.: Experiments in the transmission of *Trypanosoma hippicum* Darling with the vampire bat *Desmodus rotundus murinus* Wagner, as a vector in Panama. Jour. Prev. Med., vi (1932), 5, pp. 415-424.

[7]Clark, H. C., and Dunn, L. H.: Animal susceptibility to *Trypanosoma hippicum*, the equine trypanosome of Panama, with special reference to cattle as an unharmed host and probable reservoir of importance. (To appear in the Amer. Jour. Trop. Med.)

[8]Iturbe, Juan: Derrengadera. The effect of Bayer 205 on the *Trypanosoma venezuelense*. International Conference on Health Problems in Tropical America, p. 997. (Published by the United Fruit Company, Boston, 1924.)