

## EXPERIMENTAL STUDIES ON CHAGAS' DISEASE IN PANAMA

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The recent discovery of Chagas' disease, or human trypanosomiasis, in Panama has aroused considerable local interest. Twenty-seven years of clinical and pathological records of the Panama Canal have shown no entry of a case of Chagas' disease and the senior author's experience in field surveys for malaria in five mainland and three island countries of the Caribbean Sea during a period of three years did not reveal a case, although over 65,000 men, women and children were examined.

Nevertheless, the same character of field surveys conducted in the mid basin of the Chagres River from December 1, 1930 to August 20, 1931 have accidentally disclosed the presence of 5 human cases of Chagas' disease.

Our studies have consisted of searching for *Trypanosoma cruzi* Chagas in blood smears taken from many children during malarial surveys, examination of the blood of numerous animals for trypanosomes and subsequent inoculations to other animal hosts, and the collection of a number of *Triatoma geniculata* Latr., the arthropod believed to be the principal invertebrate host of the disease in Panama, with observations on the infection of these insects with trypanosomes.

### TRYPANOSOMAL INFECTIONS IN CHILDREN

On December 1, 1930, the first case of human trypanosomiasis or Chagas' disease, to be recognized as such in Panama was found in an infant, Eulalia Bonilla, who was admitted as a patient to the Santo Tomas Hospital, in the city of Panama. This case and two others, Theodosia Romero and José Romero, which were discovered about three months later, have been reported by Miller (1).

A fourth case was discovered on March 2, 1931. This was a child, Felix Ortega, also from the village of Aguas Buenas, where the two Romero children lived, thus making 3 cases from this village. The Bonilla child lived in a hut situated about 2 miles from Aguas Buenas. The two places from which these 4 cases came are only a short distance from the highway that extends from the Canal Zone to the site of the proposed Madden Dam on the upper Chagres River.

A fifth case was found on August 20, 1931. This was a ten-year-old colored girl, Miriam Williams. She was of Jamaican parentage but had been born in Panama City. When the Canal Zone lands were opened to settlers in 1923 this family leased and moved to a small farm along the Chiva Chiva trail about 4 miles from the main Canal Zone highway. The child has lived there since that time and has been attending the Canal Zone school at Red Tank. On August 3 she was ill with fever and unable to attend school. An eruption which the mother considered to be measles occurred and lasted about three days. The fever still prevailed when the thick blood film in which the trypanosomes of this case were found was made on August 19. There were 41 trypanosomes present in the first film. This case lived approximately 10 miles in an air line from where the other 4 cases lived. The trypanosomes demonstrating the infection in the Ortega, Romero and Williams cases were first found in thick blood films taken during routine monthly blood examinations for malaria.

#### ANIMAL INOCULATIONS FROM HUMAN CASES

Animal inoculations were made from the Bonilla and Romero cases. Rabbits, guinea pigs and white rats were inoculated with blood from the Bonilla child on the day following the discovery of her infection with trypanosomes. Thick drop blood smears from the child on this day averaged 22 trypanosomes to a slide.

*Rabbits 1 and 2.* On December 2, 1930, these two medium-sized, adult rabbits were each inoculated intraperitoneally with 0.5 cc. of blood from the Bonilla child. Both animals became positive on December 10 with trypanosomes present in moderate number in their blood on that date.

*Guinea pigs 20, 21 and 22.* On December 2, 1930, each of these 3 pigs was inoculated intraperitoneally with 0.5 cc. of blood from the Bonilla child. Guinea pigs 21 and 22 became positive on December 11 but No. 20 did not show trypanosomes in daily blood films until December 19.

*White rats 16 and 17.* On December 2, 1930, these 2 rats were each inoculated intraperitoneally with 0.5 cc. of blood from the Bonilla child. Rat 16 became positive on December 11 and trypanosomes appeared in the blood of No. 17 on December 12.

On March 13, 1931, guinea pig 79 was given an intraperitoneal injection of 1 cc. of blood taken from José Romero and guinea pig 80 received 1 cc. of blood from Theodosia Romero. Neither of these pigs ever became positive for trypanosomes. On the day the 2 pigs were injected the blood of each of the Romero cases was showing but 2 trypanosomes to a thick drop smear. These 2 cases seemed to be very light infections and the daily blood smears from them never showed more than 1 or 2 trypanosomes to a slide.

The Williams child (case 5) was brought to Gorgas Hospital on August 21, 1931, the second day after trypanosomes were discovered in her blood. On September 2, 2 guinea pigs and 1 white rat were each inoculated intraperitoneally with 2 cc. of blood taken from the patient's arm. No trypanosomes were found in the peripheral blood on this day. These 3 animals all became positive with trypanosomes present in their peripheral blood on September 22, after an incubation period of approximately twenty days. Since this article was in course of preparation at this time these animals are not included in any of the tables, totals or percentages given later in this paper and they receive no further mention in the following pages.

#### TRANSFER OF HUMAN STRAIN FROM ANIMAL TO ANIMAL

On January 15, 1931, guinea pigs 29 and 30 were each inoculated intraperitoneally with 0.5 cc. of heart's blood from guinea pig 20. At this time the peripheral blood of guinea pig 20 was showing about 22 trypanosomes to a thick smear. Guinea pig 29 became positive on February 7 and guinea pig 30 on February 12.

On February 10, 1931, guinea pigs 38 and 39 were each injected intraperitoneally with 0.3 cc. of blood from heart of guinea pig 20. Both these guinea pigs became positive, No. 38 showing trypanosomes in its blood on February 15 and No. 39 on February 27. There were about 12 trypanosomes in a thick film of blood from guinea pig 20 on the day these inoculations were made.

On January 20, 1931, a marmoset monkey, *Leontocebus geoffroyi* (Pucheran) and a five-toed armadillo, *Cabassous centralis* (Miller), were each injected intraperitoneally with 0.7 cc. of blood from guinea pig 21. The blood of this guinea pig was showing about 6 trypanosomes to a thick film on this date. The monkey became positive on February 4th but the armadillo never showed any infection.

On July 24, 1931, a juvenile (one-fourth grown) four-toed armadillo, *Dasytus novemcinctus fenestratus* Peters, was inoculated intraperitoneally with 0.7 cc. of blood from the heart of guinea pig 39. The blood of this guinea pig was showing 26 trypanosomes in a thick film on this date. This armadillo died on August 13, but since it never showed trypanosomes in its blood and its death was due to other causes this inoculation has been considered as a negative one.

Six bats, 2 *Phyllostomus hastatus panamensis* Allen and 4 *Hemiderma perspicillatum aztecum* (Saussure), that were collected in the Chilibrillo Caves and had given negative results in daily blood examinations for seven days were inoculated intraperitoneally with heart's blood from guinea pig 22 on January 21. The blood of this pig was showing an average of 5 trypanosomes to a thick film on this date. Each bat received approximately 0.3 cc. of the pig's blood. None of these bats became positive during the following twenty days and at the end of that time they were inoculated with another strain. Details of this reinoculation are given in a later page.

Five of the bats that had remained negative after inoculation with the dog strain on January 21 were reinoculated with the Bonilla strain on February 10. Each of these bats was inoculated intraperitoneally with 0.3 cc. of heart's blood from guinea

pig 20, the blood of which was showing 12 trypanosomes to a thick film on this date. Three of these bats failed to show any infection. The other two, both *Hemiderma perspicillatum aztecum* (Saussure), became positive. One, No. A. B. 5, showed a trypanosome in its blood on February 25. The second bat, No. A. B. 12, revealed 1 trypanosome in its blood on February 13 and another was found on February 25.

#### NATURAL INFECTION IN DOG

A few days following the discovery of the Bonilla case a visit was made to the house from which the child came. Blood films from two other children, one about two years and the other three years of age, were taken at this time. These films gave negative results upon examination. Several days later, on December 13, 1931, this house was again visited and blood films taken from two puppies and a kitten that were at the house. Upon our return to the laboratory these films were examined and it was found that one of the puppies was heavily infected with trypanosomes. The other puppy and the kitten were negative. On the afternoon of the same day the 3 animals were brought to this laboratory. The puppies appeared to be about three or four months old and were greatly emaciated and weak. The kitten was in the same poor condition as the puppies and this probably was due as much to insufficient food as to other causes. Thick blood films from the infected puppy, No. 1, showed about 150 trypanosomes to a slide at this time. Both of these puppies died on December 15, two days after being brought to the laboratory. The kitten was kept under observation for several weeks but no trypanosomes were found in its blood during this time.

From the literature at hand we are unable to find any previous report of a natural infection of Chagas' disease in a dog and we believe that this is the first instance of the kind.

#### TRANSFER OF DOG STRAIN TO OTHER ANIMALS

On December 14, 2 white mice, 2 white rats and 2 guinea pigs were each inoculated with blood taken from the jugular vein of infected puppy 1. The mice and rats each received 0.5 cc. of

blood and the guinea pigs were each given 1 cc. All 6 animals became positive and showed trypanosomes in their blood. One mouse was positive in ten days and the other in sixteen days. The 2 rats became positive in nine and eleven days and the guinea pigs in three and four days.

On January 21, 2 guinea pigs were each inoculated intraperitoneally with 1 cc. of blood from the heart of white rat 22. This rat was one of those infected through inoculation with blood from puppy 1. These 2 guinea pigs showed trypanosomes in their blood in fourteen and fifteen days after inoculation.

Three bats, *Hemiderma perspicillatum aztecum* (Saussure), that had shown no natural infection of trypanosomes in their blood during seven days after capture were each inoculated with 0.6 cc. of blood from the heart of white rat 22 on January 21. There were 5 trypanosomes present in a thick film of blood from the rat on this date. Two of these bats never showed any infection. One trypanosome was found in a blood film from the third bat, No. A. D. 6, on February 10.

#### NATURAL INFECTION IN ARMADILLOS

Chagas (2) and Torres (3) have found trypanosomal infections in the armadillos in Brazil. The trypanosomes present in these animals appeared to be indistinguishable from *Trypanosoma cruzi* and have been accepted as being that organism. Three species of the Brazilian armadillos appear to acquire this through natural infection. Chagas (4) found that from 46 to 50 per cent of the species, *Dasypus novemcinctus*, were infected. This species differs from our common armadillos, *Dasypus novemcinctus fenestratus* Peters, only in subspecific differences and through geographic distribution. We were fortunate in being able to find that a number of our Panama armadillos were also infected with a trypanosome morphologically similar to those found in the human cases and we have no hesitancy in recording it as *T. cruzi*.

During the past thirteen months a total of 28 armadillos, 25 *Dasypus novemcinctus fenestratus* Peters and 3 *Cabassous centralis* (Miller), have been received alive at this laboratory. The blood

of twenty-four of these animals was examined daily by the thick film method during a period of seven or more days. The other four died or were killed in less than seven days, two being sacrificed three days after being received and two died on the fifth day. Six, or 21.4 per cent, of these animals, all *Dasypus novemcinctus fenestratus*, were found to be infected with trypanosomes.

*Armadillo 1.* Received on August 12, 1930. Had been captured near Arraijan. A trypanosome was found in one of its daily blood smears on August 20, eight days after being received. The animal died on August 21. Smears of heart's blood taken at autopsy showed trypanosomes. The infection in this armadillo was found before the first case of human trypanosomiasis was discovered in Panama.

*Armadillo 18.* Was captured near Patuga, in the Province of Herrera, and was brought to the laboratory on July 1, 1931. One trypanosome was found in its blood on July 5. It was sacrificed on July 9. A thick film of post-mortem blood was positive for 2 trypanosomes.

*Armadillo 20.* This animal was received on July 14 from Parita, Province of Herrera. A thick film of its blood taken on July 15 contained 15 trypanosomes. A gradual increase in the number of trypanosomes in the blood seemed to occur until ninety-nine were present in a thick film taken on July 23. Its post-mortem blood showed many trypanosomes in both thick and thin films.

*Armadillo 21.* Received on July 14 from Parita. A thick blood film taken from this animal on July 15 contained 25 trypanosomes. This number increased until seventy-five could be found on July 21. Many trypanosomes were present in the post-mortem blood of this animal after it was killed on July 23.

*Armadillo 23.* Captured near Parita and brought to the laboratory on July 14. One hundred and fifty trypanosomes were found in a thick film of its blood on July 15, one or two being present in each microscopic field. This animal was killed on July 23. Trypanosomes were present in each microscopic field of the thick film of blood taken from the animal's heart at autopsy.

*Armadillo 25.* This animal was brought to the laboratory on

August 8. It had been captured near Patuga. A thick blood film taken from this armadillo on August 10 showed 6 trypanosomes. This number increased to thirty-one to a film by August 16. This armadillo died on August 18. Blood films taken at autopsy showed 52 trypanosomes to a thick film and three to a thin film.

It was interesting to note that of the positive armadillos only one could be considered an adult. This was No. 18 with a comparatively light infection. The other five were juveniles and apparently not more than one-quarter grown. Three of the latter were part of a batch of five that were captured near Parita and since the five were about the same size they probably all belonged to the same litter. The other two of the batch showed no trypanosomes.

The two villages of Parita and Patuga, in the Province of Herrera, where five of the positive armadillos were caught, are hardly more than 4 miles apart.

The daily increase of the trypanosomes in the blood of four of these juvenile armadillos following their capture was rather striking and leads one to wonder regarding the cause of it. It may have been due to an effect produced on the animal through being handled, lack of proper food or by other abnormal conditions caused by their captivity.

The foregoing observations on the infection in armadillos cause us to suspect that a great many of the armadillos in Panama become infected with trypanosomes while in the early juvenile stage and as they become older the infection is either thrown off entirely or the trypanosomes become so few in number that they are seldom found in the peripheral blood unless something abnormal happens to the animals and causes a relapse.

#### ATTEMPTS TO TRANSFER ARMADILLO STRAIN TO OTHER ANIMALS

The only attempts made to transfer the armadillo strain of trypanosomes to other animals was the inoculation of 2 guinea pigs with blood from the heart of armadillo 1 on August 21, 1930. This blood was taken while the armadillo was in a moribund condition. Each pig received 0.5 cc. of the blood intraperito-



neally. The pigs failed to show any positive results. The infection in this armadillo was a very light one.

#### NATURAL INFECTION IN OPOSSUMS

In 1929, Robinson (5) found an opossum, *Didelphis marsupialis*, naturally infected with a trypanosome that seemed to be *T. cruzi*, near Tela, Honduras. This appears to be the first report of a marsupial being infected with this trypanosome. A number of naturally infected local opossums of practically the same species were also found by us during our investigation. The trypanosome present in the blood of these animals resembled *T. cruzi* and we believe with Robinson, that it should be so recorded.

During the past seven months, 81 opossums, *Didelphis marsupialis etensis* Allen, were received at this laboratory and the blood of each one was examined. Owing to the existing conditions the number of daily examinations of each varied considerably. Twenty-five of the animals had been injured during capture and died or were killed so soon after arrival at the laboratory that only post-mortem blood was examined. Blood films for examination were taken from two on one day only, from twelve on two days, nine on three days, eleven on seven days, three on eight days and nineteen for periods ranging from eight to ninety days.

A total of twenty, or 24.6 per cent, of the 81 opossums examined was found to be infected with trypanosomes. The number present in the blood of these animals varied considerably and ranged from one to eighty-eight to a thick film. The greater number of the animals, however, showed only light infections.

Trypanosomes were found in blood films from eight of the animals that were killed on arrival and whose post-mortem blood only was examined. Of the others that remained alive one day or longer and which were found to be infected, 5 showed trypanosomes on the first day, 3 on the third day, 1 on the tenth day and 1 animal did not show a positive film until the twenty-first day after its arrival at the laboratory.

The infected opossums came from several locations. One was captured in the Chilibrillo Caves, about 1½ miles from Aguas

Buenas. Another came from Summit, in the Canal Zone. Two were taken near Alhajueta, about 5 miles from the Chilibrillo Caves. Ten were captured near Parita and six were brought from Patuga. Five of the positive animals were found in one lot of 14 opossums received from Parita on July 8, 1931.

#### NATURAL INFECTION IN BATS

Wenyon (6) informs us that bats naturally infected with trypanosomes have been found by various workers in Europe, India, Africa, South America, the West Indies and other parts of the world. Although some of the trypanosomes found in these bats closely resembled *T. cruzi* they, nevertheless, were believed to be different species.

The trypanosomes found in the bats of Panama are morphologically similar to *T. cruzi* and in our estimation should not be recorded as a separate species. There seems no reason to believe that bats are likely to have a trypanosome limited to the Chiroptera or that it would be incongruous for the latter to be infected with *T. cruzi*.

Therefore, as a result of our findings during these investigations, we feel quite confident that the trypanosomes found by us in the bats of Panama were *T. cruzi*. This conviction is due not wholly to the similarity of morphology of the trypanosome but also to the other circumstances involved. We found an infected opossum, many infected bats and numerous infected *Triatoma* in the same chamber of a cave and this cave was only a comparatively short distance from the habitations of the human cases. In addition, the trypanosome of the bat was transmitted to laboratory animals and we look upon this as evidence of considerable weight.

The blood of 161 bats have been examined during the past thirteen months and a total of thirty, or 18.6 per cent, was found to be infected with trypanosomes. The species represented by those examined and the number of each that were found infected are given below:

*Hermiderma perspicillatum aztecum* (Saussure). Seventy-five bats of this species were examined and eighteen, or 24 per cent,

were found to be infected with trypanosomes. Fifty-five of those examined, including sixteen positives, were collected in the Chilibrillo Caves and twenty-one, with two positives, were taken from culverts beneath the highway at Bella Vista, suburb of Panama.

*Phyllostomus hastatus panamensis* Allen. Thirty-seven of these large bats were collected in the Chilibrillo Caves. Upon examination, eight, or 21.6 per cent, were found to be positives.

*Uroderma bilobatum* Peters. Fifteen of this species that were found hanging in trees at Summit were examined and one, or 6.7 per cent, showed trypanosomes in its blood.

*Glossophaga soricina leachi* (Gray). Thirteen of this species were examined. Nine of these were found in a small detached building used for a private garage near a dwelling at Bella Vista and four were captured in a culvert beneath the roadway on the west side of the Canal, nearly opposite the Miraflores Locks. One of the number taken at Bella Vista proved to be positive, giving 7.7 per cent of positive findings for this species.

*Artibeus jamaicensis jamaicensis* Leach. Eight specimens of this robust bat were captured in palm trees at Summit. Two or 25 per cent, were positive.

*Molossus sinaloae* Allen. Thirteen of this species were collected in the attic of a house at El Real, in the Tuira River district. None in this lot was found to be infected.

One hundred and five of the 161 bats examined were chloroformed soon after their arrival at the laboratory and a thick and a thin film made from the blood of each one. The blood was obtained either by clipping the tip of an ear or drawing it directly from the heart with a hypodermic syringe. The bats were then usually killed in order that all ectoparasites could be collected. This resulted in the examination of but one thick film from each bat that was handled in this way. The remaining fifty-six bats were kept alive for some time for observation or experimental purposes and this allowed them to be examined daily over periods ranging from two to fifteen days or longer. Trypanosomes were found in twenty-three, or 76.6 per cent, of the infected bats during the examination of the first film from each. The infections in the

other seven positives were discovered during later examinations that ranged from two to six days.

The number of trypanosomes present in the first positive slide taken from each infected bat varied from a single trypanosome to four hundred or more in a microscopic field of a thick film. One lot of thirty-four *Hemiderma perspicillatum aztecum* that was collected at one time in the Chilibrillo Caves contained twelve positives. These infections were all found during the examinations of the first films. Several of this lot showed heavy infections, one having more than four hundred in each microscopic field.

We do not consider it presumptuous to express the belief that the majority of the bats of Panama become infected with this trypanosome in early life and that many of them spontaneously recover to become chronic carriers of the parasite.

#### TRANSFER OF BAT STRAIN TO OTHER ANIMALS

Animal inoculations were made with the strain of bat trypanosomes and the infection was transmitted to white mice, white rats, guinea pigs and dogs.

*White rats 1 and 2.* On August 5, 1930, seven bats, *Artibeus jamaicensis jamaicensis*, were captured in a palm tree at Summit and brought to the laboratory. Two of these were adult females and five were juveniles, three males and two females, that apparently were slightly more than half grown. Approximately 0.3 cc. of blood was taken directly from the heart of each of the two adults and two of the juveniles and injected into the peritoneal cavity of rat 1. A second rat, No. 2, was inoculated in the same manner with blood from the other three juvenile bats. Blood films taken from each of these bats showed an infection with trypanosomes in one of the adults. All of the others gave negative results. Rat 1 died August 16 before exhibiting any signs of infection. No. 2 became positive and showed trypanosomes in its blood on August 20, fifteen days after inoculation.

*White rat 8.* On August 23, rat 8 was inoculated intraperitoneally with 0.5 cc. of blood taken from the heart of infected rat 2. There was but one trypanosome to be found in a thick film of

blood from the latter rat on this date. Rat 8 became positive and showed trypanosomes in its peripheral blood on September 6, after an incubation period of fourteen days.

*Guinea pig 5.* This pig was also inoculated with 0.5 cc. of heart's blood from rat 2 on August 23 and became positive on September 1.

*White mice 18 and 19.* On December 8 these 2 mice were each inoculated with 0.5 cc. of blood from the heart of guinea pig 5. The last date that trypanosomes had been found in the peripheral blood of this guinea pig was November 17 twenty days previously. Since that date the pig's blood was examined six times at intervals of from three to five days between examinations. No trypanosomes were found in these examinations and none were present in a blood film taken on the date of these inoculations. Both of these mice became positive for trypanosomes on December 22 and developed fairly heavy infections.

*Guinea pig 25.* This guinea pig was also injected with 1 cc. of blood from guinea pig 5 simultaneously with the inoculations of mice 18 and 19. Trypanosomes appeared in its blood on December 17.

*Dogs 3 and 4.* These dogs were about two months old and had been well cared for and were fat and in good condition. No 3 weighed  $9\frac{1}{2}$  pounds and No. 4 weighed 11 pounds. They were both inoculated on January 17. No. 3 received 1 cc. of blood from the heart of guinea pig 25. No. 4 was given 0.5 cc. of blood taken from the heart of white mouse 18. Both of these inoculations produced positive results. Trypanosomes were found in the blood of dog 3 on February 5 and of dog 4 on January 27.

Wenyon (7) states that "Battaglia (1914) has claimed that *T. vespertilionis* is pathogenic to rabbits. He makes a similar claim for *T. lewisi*. No other observer has succeeded in confirming these statements, attempts at infecting laboratory animals with the trypanosomes of bats having invariably failed."

It may be noted from our success in infecting laboratory animals with the trypanosome of the bat that in Panama this organism is readily transmissible.

## NATURAL INFECTION IN A SQUIRREL

A half-grown squirrel, *Sciurus gerrardi morulus* Bangs, had been kept as a pet for some time in the household of the Romero family, the two children of which were found to be infected with Chagas' disease on March 9. On March 30 this squirrel was brought to the laboratory and blood films taken from it for examination. A search of the first thick film showed eight trypanosomes. Daily blood films showed from two to six trypanosomes to a film until April 4 when the animal was placed on the survey list to be examined only at ten-day intervals. The examination of April 14 showed two trypanosomes to a film and on April 24 one was present to a film. This was the last date that the blood of the squirrel was found to be positive, although it was examined once every ten days during the subsequent four months.

The trypanosome in this squirrel was similar in morphology to *T. cruzi* and we have accepted it as such. We believe that it may have acquired its infection at approximately the same time as the 2 human cases in the house where this squirrel was secured.

## INSECT VECTORS OF CHAGAS' DISEASE IN PANAMA

The insects that have been incriminated in the transmission of Chagas' disease in South and Central America belong to the family Reduviidae. This family is represented in Panama mainly by two members, *Triatoma geniculata* Latr. and *Rhodnius prolixus* Stal. Other species have been reported from this Republic but apparently they have been found only in small numbers. Neither of the two species that are commonly found here are present in abundance although they may be numerous in certain small areas.

Specimens of *Rhodnius prolixus* have been received from time to time by the junior author during a period of more than twenty years, but always in very sparse numbers. Three lots of from two to four each have been received from Capira. These were collected by a local resident who was engaged to search for them. Occasional specimens have been found at Ancon, in the Canal Zone, at Canete, in the Bayano River region and at Santa Rosa

and San Juan, in the Chagres River valley. Two males of this species were also sent to this laboratory by the Bonilla family, one member of this family was the first human case of Chagas' disease to be found here. The two bugs were captured in or near the hut in which this family lives. Since we have not been able to secure *R. prolixus* in any numbers no studies have yet been carried out to determine their possible infection with trypanosomes in Panama.

In Brazil the *Triatoma geniculata* is found in the burrows of armadillos and it seems to be the belief there that these animals receive their infection from the bugs. If such is the case the infection is probably passed back and forth from the infected bugs to young animals and from the infected animals to bugs newly emerged from the eggs. Since we have not had opportunities for examining the burrows of armadillos in Panama we are unable to state whether or not they are infested with these bugs in this part of the world.

Only two specimens of *Triatoma geniculata* had been collected by us in Panama previous to the beginning of these studies in Chagas' disease. Both of these were taken from the outside of a house screen, one at Ancon and the other at Balboa, in the Canal Zone, during 1914. During the past few months we have also received two specimens from Santa Rosa and one from Aguadulce. The main source of our supply of *T. geniculata* that were used in these experiments was the Chilibrillo Caves that have been previously mentioned in the foregoing pages as a place where an opossum and numerous bats infected with trypanosomes were found.

#### THE REDUVID BUG, *TRIATOMA GENICULATA*, IN THE CHILIBRILLO CAVES

The Chilibrillo Caves are located in the extensive limestone ridge sloping along the Chilibrillo River, a tributary of the Chagres' River. They are hardly more than  $\frac{1}{2}$  mile from the new Madden highway that extends from the Canal Zone to the upper Chagres River. In air lines from these caves it is about 1 mile to the Bonilla house,  $1\frac{1}{2}$  miles to Aguas Buenas, 5 miles to Santa

Rosa, 7 miles to Summit in the Canal Zone and 15 miles to Panama City.

The easily accessible parts of these caves consist of three large chambers and numerous tunnels, many of the latter being connected. The central chamber is a fairly dry, flask-shaped room approximately 185 feet in length and increasing from 20 feet in width near the entrance to 50 feet wide near the posterior wall. The roof is nearly hemispherical and at the highest part is hardly more than 12 feet from the floor of the cave.

At the beginning of the present year it was planned to carry out a rather complete survey of the fauna of these caves. This work was begun on January 15 and during the subsequent ten weeks Mr. A. L. Brody, Assistant Entomologist at this laboratory, accompanied by a West Indian boy, spent an average of three forenoons each week in collecting specimens of the fauna present in various part of the caves.

Bats of several species were present by the thousands and were hanging, either singly or in large groups from the roof and walls of the caves.

A total of ninety specimens of *Triatoma geniculata* was taken in these caves. Eighty-nine of these were collected in the central chamber described above, only one being found in the other parts of the caves. Eighty-five were taken in a small alcove on one side of this chamber about 50 feet from the entrance. The infected opossum, No. 1, was also found in this alcove.

The bugs were not found in abundance. From one to seven could be located on the walls each time the cave was visited. Seven was the largest number taken at one visit. Each visit represented approximately two and a half hours of searching by two collectors with the aid of electric flashlights. This leads us to believe that the bugs remain hidden within the numerous small crevices present in the walls of the cave during the greater part of the time and they are not seen on the walls except when they emerge in search of a meal of blood.

These bugs readily fed on bats in the laboratory and it is quite probable that under natural conditions in the caves they commonly obtain their blood meals from the bats as well as from



other animals that may be present. Three opossums, however, were the only mammals other than bats seen in the caves during this survey.

Nine of the *Triatoma* collected were adults. The other eighty-one were immature forms that ranged from unfed larvae to last stage nymphs.

A report by Caudell (8) on insects collected in these caves in 1923 by Shannon, Zetek and Molino shows that specimens of *T. geniculata* were found there at that time. In view of this it is quite probable that this species of *Triatoma* has been present in the caves for many years.

#### TRYPANOSOMAL INFECTION IN TRIATOMA GENICULATA

Studies to determine the number of the *Triatoma* that might be infected with trypanosomes were carried out on the specimens collected in the caves. These studies consisted of (a) examination of the feces of the bugs, (b) inoculation of guinea pigs with macerated bugs and (c) feeding the bugs on guinea pigs.

##### *Examination of the feces*

Microscopic examinations were made of smears of feces from eighteen of the bugs. The smears were made from the material excreted soon after taking a meal of blood from a guinea pig. As a bug became replete and was removed from the guinea pig it was placed in a clean, short, wide-mouthed bottle. Excreta was usually discharged within a half hour after a bug was placed in the bottle and one or two slides was then prepared from this material. Trypanosomes were found in slides from ten out of the eighteen bugs, giving a result of more than 55.5 per cent of positives in the lot. Had smears been made and examined on more than one occasion from each bug it is probable that more positives would have been discovered.

##### *Inoculation of guinea pigs with macerated Triatoma*

Twenty-six of the bugs, representing the different nymphal stages as well as adults, were used in these inoculation experiments. Each bug was well macerated in a small amount of

normal saline solution and the resultant emulsion injected into the peritoneal cavity of a guinea pig. This was done either on the day the bugs were collected or on the day following and without giving them any chance to feed while at the laboratory.

Five of these bugs had round abdomens and upon maceration each showed blood that appeared to be of fairly recent ingestion in the stomach or intestinal tract. Three were partly depleted and the contents of their alimentary canals were fairly well digested. Eighteen were quite flat and all material present in the intestines was thoroughly digested.

Twenty-one, or 80.8 per cent, of the 26 guinea pigs that were inoculated became positive, showing that the greater number of the *Triatoma* found in the cave were infected with trypanosomes.

Nothing of any importance could be noted in regard to the relation of the state of repletion of the bugs to their infectivity. One of the five nearly replete bugs gave negative results. Each of the three that were partly replete proved to be positive. The eighteen flat ones gave fourteen positives and four negative results.

#### *Feeding Triatoma on guinea pigs*

A number of the *Triatoma* were kept for use in life history studies that we began to carry out with this species.

In these studies each of the bugs was given an opportunity to feed on a guinea pig at intervals of every four days while in the immature stages and from two to four days for the adults. They were allowed to feed by placing each one in the bottom part of a small, round, cardboard pill box and the latter inverted on the shaven area of the abdomen of the guinea pig. The guinea pig was previously immobilized by bandaging it to a narrow strip of wood. A few turns of the bandage also served to hold the pill box firmly in place and prevent the escape of the bug. This arrangement placed the bug directly on the skin of the guinea pig with an opportunity for feeding in the dark. Three or four boxes, each containing a bug, were usually bandaged on a guinea pig at one time. Each box was left on the guinea pig from thirty to forty minutes.

Thirty-five bugs were used in these studies when they were started early in February. The mortality in this group was high and this number gradually decreased until but eleven were left at the end of June.

Each guinea pig was used as a host for a period of from four to six days. The total number of bites received by each guinea pig as well as the number of bugs that fed on each varied considerably, this depending entirely on the appetites of the bugs. Twenty-eight bites was the largest number definitely known to be received by one animal while seven was the smallest. None of the guinea pigs was bitten by all of the bugs during the period that it was used. Several of the bugs, however, fed two or more times on one animal. A positive bite was determined by the appearance of the bug when removed from the host. If much blood had been ingested the effect of it was easily noticeable in the color, swelling, etc., of the abdomen of the bug. There was no way of determining, however, the number of times that a bug might have inserted its mouth parts in the skin and withdrawn them without feeding.

Daily blood examinations were made on the first 15 guinea pigs that were consecutively used in these studies. Seven, or 46.7 per cent, of these became positive and showed trypanosomes in their peripheral blood. The greatest number of bites to be received by one of the animals that became positive was twenty-two and the least number was eight.

In going over the literature on Chagas' disease and *Triatoma* one finds that a question exists in regard to whether the transmission of the disease to man or animal occurs through the bite of the bug or through contamination with feces that it deposits while feeding. In our studies the bugs were given the opportunity of resting directly on the skin of the host while feeding which would of course be nearly as favorable for infection by means of the excreta as by the bites alone. Feces were occasionally noted on the skin, especially when a bug became greatly engorged and was left on the host for longer than thirty minutes. More frequently, however, the bugs did not discharge excreta until after they had been replaced in the bottles in which they

TABLE 1

*Incubation period of T. cruzi in animals infected from various sources*

SOURCE OF INOCULATION	ANIMAL INOCULATED	LABORATORY ANIMAL NUMBER	INCUBATION PERIOD
			<i>days</i>
Human case 1 (Bonilla) .....	Rabbit 1		8
	Rabbit 2		8
	Guinea pig	20	17
	Guinea pig	21	9
	White rat	16	9
	White rat	17	10
Guinea pig 20 (first subinoculation) ..	Guinea pig	29	23
	Guinea pig	30	28
	Guinea pig	38	5
	Guinea pig	39	17
	Bat A. B.	5	15
	Bat A. B.	12	3
Guinea pig 21 (first subinoculation) ..	Marmoset monkey	58	15
Dog 1 (natural infection) .....	White mouse	20	16
	White mouse	21	10
	White rat	22	9
	White rat	23	11
	Guinea pig	26	4
	Guinea pig	27	3
White rat 22 (first subinoculation) ..	Guinea pig	33	14
	Guinea pig	34	15
	Bat A. D.	6	20
Bats (pooled blood from four) .....	White rat	2	15
Rat 2 (first subinoculation) .....	White rat	8	14
	Guinea pig	5	8
Guinea pig 5 (second subinoculation) .....	White mouse	18	14
	White mouse	19	14
	Guinea pig	25	9
Guinea pig 25 (third subinoculation) ..	Dog 3		19
	Dog 4		10

were regularly kept. We have, therefore, no way of determining the manner in which the infection occurred in our pigs that became positive. It is planned to carry out investigations on this problem of transmission at a later date.

#### INCUBATION PERIOD OF *TRYPANOSOMA CRUZI* IN ANIMALS

The incubation period of *T. cruzi* varied considerably when inoculated into animals. This variation was not due entirely to the strain being changed through numerous transfers since marked differences are shown in the first inoculations. The incubation periods in each animal that became positive from being inoculated

TABLE 2

*Incubation period of T. cruzi in guinea pigs inoculated with macerated Triatoma*

GUINEA PIG NUMBER	INCUBATION PERIOD	GUINEA PIG NUMBER	INCUBATION PERIOD
	<i>days</i>		<i>days</i>
40	27	57	7
42	12	59	18
44	7	61	17
45	11	62	22
46	27	63	24
48	9	67	15
49	10	68	23
52	13	71	14
53	10	76	6
55	17	78	13
56	10		

with infected blood or were infected through experiments with the *Triatoma geniculata* are shown in table 1.

The same wide variation in the incubation period shown in the animals inoculated with infected blood was also manifested in the guinea pigs inoculated with macerated *Triatoma* (table 2).

It was impossible to definitely determine the incubation periods in the 7 guinea pigs that became infected through feeding the bugs on them since each guinea pig was used as a host for the bugs on from four to six consecutive days. Taking the first and last days on which a pig was used as the maximum and minimum possible time between infection and the appearance of try-

panosomes in the peripheral blood, the shortest period was from six to ten days and the longest from eighteen to twenty-two days.

#### THE DEGREE AND PERSISTENCE OF INFECTION IN VERTEBRATE HOSTS

Our observations on the infection of *T. cruzi* in vertebrate hosts were carried out mainly on the laboratory animals that were experimentally infected during these studies. The majority of the armadillos, opossums, bats, etc., seldom lived more than a few weeks after they were received at the laboratory. Many were killed for intestinal examinations in connection with other investigations that were being carried on at the time. Others died through causes due to previous injuries, improper diet, confinement, etc. The daily handling of them to secure blood for films was also probably inimical to their longevity under laboratory conditions. Furthermore, since the naturally infected animals were positive before they were received there was no way of determining the length of time the infection had persisted before coming under our observation. The small amount of information that we did acquire on the infection in wild animals is given in the previous pages.

The source of the infection in laboratory animals seemed to have but little bearing on the intensity of the infection. There was comparatively little difference to be noted in infections produced through inoculations with positive blood, macerated bugs or by the bugs feeding upon the animals.

Daily blood examinations were made on nearly all the experimentally infected animals during a period of from six to twelve weeks following the appearance of trypanosomes in the blood. After that period the examinations were carried out only at ten-day intervals so long as the animal lived. The thick blood film method was used in these examinations and since the films were made daily by the same person they remained fairly regular with regard to the amount of blood used for each film. These examinations gave a good opportunity for observing the variations occurring in the number of trypanosomes found from day to day, not only between different animals but also the daily changes that took place in individual animals.

The largest number of trypanosomes found in a film during the course of an infection in an animal constituted what we are terming the "peak peripheral infection" and the day on which this peak infection occurred as the "infection peak day." The number of trypanosomes present at the peaks of peripheral infections varied considerably among the animals and ranged from one to more than sixteen hundred. There was also a wide variation shown in the number of days elapsing between the date that trypanosomes were first found in an animals' blood and infection peak day, the shortest time being three days and the longest one hundred and fifteen days.

The 21 guinea pigs that became positive through inoculation with macerated *Triatoma* seemed to be somewhat more consistent in having large numbers of trypanosomes at peak peripheral infections than those in the other experimentally infected groups. One of these guinea pigs ran a very light infection and never showed more than 1 trypanosome to a film and positive films were found only on occasional days. The heaviest infection in this group had more than 600 trypanosomes at the peak peripheral infection. The number of trypanosomes present at the peaks of peripheral infections of the other 19 guinea pigs of this lot when arranged in groups show that two had less than 100 each, three between 100 and 200, two between 200 and 300, eleven between 300 and 400 and two had between 400 and 500 each. Among these guinea pigs there were thirteen that reached their infection peak days in less than twenty days after trypanosomes first appeared in their blood, five in from twenty to thirty days, two in from thirty to forty days and one did not reach the peak until one hundred fifteen days after first becoming positive. This last animal had a comparatively light infection with a peak peripheral infection of 43 trypanosomes.

Two of the guinea pigs that became positive through the bugs feeding upon them never showed more than one trypanosome to a film. The peak peripheral infections of the other five ranged from 150 to more than 300 trypanosomes. These five all reached their infection peak days in from twelve to eighteen days after becoming positive.

The heaviest infection of all the animals was a peak peripheral infection of more than 1600 trypanosomes at twenty-one days after the beginning of the positive infection, which occurred in a guinea pig inoculated with blood from the naturally infected dog 1. The second high peak peripheral infection had more than 1000 trypanosomes after an interval of seventeen days from the first positive day. It was in a guinea pig that was inoculated with blood from human case 1. Among the other animals of this blood inoculated group there were three that showed only one trypanosome to a film. About half of the others had a peak peripheral infection of less than 100 trypanosomes. Taken as a whole this group had a much lower average peak than the

TABLE 3

*Length of infectivity periods in animals and number of animals still infected*

NUMBER OF DAYS OF INFECTIVITY PERIODS	NUMBER OF ANIMALS	NUMBER OF ANIMALS STILL INFECTED
0-25	3	
25-50	6	
50-100	11	2
100-150	15	1
150-200	10	2
200-250	1	
250-300	3	2

guinea pigs infected by means of the *Triatoma*. In this blood inoculated group, which represents rabbits, guinea pigs, white rats, white mice and bats, the guinea pigs ran the heaviest infections. One white mouse reached its peak of 78 trypanosomes after one hundred ten days, while another had a peak of 26 trypanosomes after forty-one days. The heaviest infection in a white rat was 303 trypanosomes after thirteen days of infection. The two rabbits reached the respective peaks of 23 and 68 trypanosomes, one in ten days and the other in thirteen days.

The persistence of infectivity in the 55 experimentally infected animals (exclusive of the 3 bats) varied from one to two hundred seventy-eight and more days, since the animal in which this maximum infection period is still infected at the time of writing.



Three of these animals died before the trypanosomes had disappeared from their peripheral blood and three more were sacrificed while still showing the parasites. The periods of infectivity, or the time elapsing between the date of finding the first trypanosomes in the peripheral blood of an animal and the date the last one was noted, have been placed in groups according to the length that the infectivity period of each animal persisted. The number of days in these infectivity periods and also the number of animals that are still showing trypanosomes in their blood streams are shown in table 3.

Various opinions have been given by workers on Chagas' disease in regard to the susceptibility of infection of different animals. There also seems to be a belief that very young animals are much more susceptible than are adults. In our work with this disease we found that guinea pigs proved to be the most satisfactory laboratory animals. All the other laboratory animals that are commonly used were easily infected and most of them ran fairly heavy infections. We found adult animals to be so susceptible to this disease that we had no reason for using very young ones.

#### PATHOGENICITY OF CHAGAS' DISEASE

Our studies on Chagas' disease have been carried out over too short a period and have been of too superficial a nature to enable us to gain much information of the pathogenicity of *T. cruzi*. None of the 5 human cases, however, appeared to present any very definite symptoms that could be directly ascribed to this disease. The trypanosomes disappeared from the peripheral blood of these 5 cases without specific treatment. If the disease is of a chronic nature it may be that the trypanosomes will appear again in the peripheral blood at intervals.

No serious results developed in the animals infected, either naturally or experimentally, that came under our observations. Of the 39 guinea pigs that were experimentally infected there are 33 still living. The length of time that has elapsed since finding the first trypanosomes in the blood of each of these 33 guinea pigs and the date of this writing varies from sixty-six to two

hundred seventy-eight days. Only two have periods of between sixty-six and one hundred days, one has one hundred thirty-three days, thirteen have between one hundred fifty and two hundred days, fourteen between two hundred and two hundred fifty days and there are three between two hundred seventy and two hundred eighty days. The guinea pigs showing the two highest peak peripheral infections of 1600 and 1000 trypanosomes are still alive after the respective periods of two hundred six and two hundred seventy-eight days. The one with the 1000 peak is still showing trypanosomes. The other one with the 1600 peak has been giving negative films during the past seventy-two days. Of the two rabbits inoculated one is still alive two hundred seventy-nine days after its first infected day. One white rat out of six that were infected is still living after two hundred seventy-seven days; one of the other five was sacrificed. It would appear that a much larger percentage of these animals should have died before this date if the trypanosome possessed a high degree of virulence.

Examinations of sections of heart muscle from an armadillo, a dog and an opossum revealed the presence of leishmania forms of this trypanosome but extended searches were necessary to locate them. These animals had all shown the trypanosomes in their blood. Many animals with positive blood films have failed to show leishmania forms in the heart muscle. Perhaps the infection is completely eradicated in many cases. The heart muscle is not invaded in the striking manner that is noted in sarcosporidiosis. This form of trypanosomiasis seems to belong to animals that live in dens or burrows rather than to other animal species or to man.

The region in which the human cases were found had been recently cleared and placed under cultivation. As a result nearly all burrowing animals had been killed for food or to prevent destruction of crops. This, no doubt, left the insect vector without its normal host for blood meals and forced it to meet compulsory conditions which meant feeding on other animals. The human being is probably not the host of choice.

## SUMMARY

1. Five cases of Chagas' disease have been found in children in Panama during the past ten months.

2. Rabbits, guinea pigs and white rats were inoculated and became positive with heavy infections from one of these human cases. This strain was successfully transferred to a monkey, guinea pigs, white rats, white mice and bats.

3. A young dog naturally infected with trypanosomes that appear to be identical with those found in the human cases was discovered at the home of the first human case.

4. Guinea pigs, white rats and white mice were infected through inoculation of blood from a naturally infected dog. This strain was carried on to other guinea pigs and a bat.

5. Twenty-eight armadillos, *Dasypus novemcinctus fenestratus* Peters were examined and six, or 21.4 per cent, were found to be infected with a trypanosome that we believe to be *T. cruzi*.

6. Eighty-one opossums, *Didelphis marsupialis etensis* Allen, were examined and twenty or 24.6 per cent were found infected with what was apparently *T. cruzi*.

7. A squirrel, kept as a pet in the house where two cases of Chagas' disease lived proved to be infected with a trypanosome identical with *T. cruzi*. This was the second occasion in which a pet, in the same household with one of the five human cases, was found positive for trypanosomes.

8. Bats infected with a trypanosome believed to be *T. cruzi* were found in Panama. A total of 161 of these flying mammals was examined and 30, or 18.6 per cent proved to be infected. Those found positive represented 5 species.

9. The trypanosome of the bats was readily transmitted to dogs, guinea pigs, white rats and white mice.

10. Ninety specimens of *Triatoma geniculata* were collected in the Chilibrillo Caves. This completes a record of infected *Triatoma*, infected bats and an infected opossum found in the same chamber of these caves.

11. The feces of 55.5 per cent of the bugs proved to contain trypanosomes.

12. Twenty-six guinea pigs were each inoculated with a macerated *Triatoma* and 21, or 80.8 per cent, became positive.

13. Seven guinea pigs became infected through *Triatoma geniculata* feeding upon them. It was not determined whether these infections were caused through the bites of the bugs or through infection with their feces.

14. The incubation period of the experimentally infected animals varied from three to twenty-eight days.

15. The degree of the infection in the experimentally infected animals varied considerably. One guinea pig had a peak peripheral infection of more than 1600 trypanosomes in a thick blood film.

16. The persistence of infectivity has extended in 1 guinea pig for more than two hundred and seventy-eight days.

17. Although all our laboratory animals could be infected with this trypanosome the guinea pig seemed to be the most satisfactory for experimental purposes. The adult animals proved so susceptible to infection that no very young ones were used.

18. The trypanosomes soon disappear from the peripheral blood of the human cases. Infected animals have lived for a considerable number of days since the beginning of their infection and in some of them trypanosomes have been found for many days.

19. Leishmania forms of the trypanosome have been found in the hearts of some animals but others fail to show them.

20. Our experience indicates that this trypanosome does not possess a high degree of virulence. However, we cannot read what the future holds in store in the way of remote serious sequelae.<sup>1</sup>

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