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THE CATTLE RESERVOIR FOR EQUINE TRYPANOSOMIASIS IN PANAMA

ADDITIONAL NOTES ON THE SUBJECT

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A previous report (1) by our institution with special reference to cattle as an unharmed host and probable reservoir for *Trypanosoma hippicum*, the cause of our local equine trypanosomiasis, has been given further study. It was learned, after our report was published, that a few Venezuelan cattle were in that herd of 135 Panamanian cattle. All of these were on the same range with a herd of horses and mules that had suffered an epidemic of trypanosomiasis. Since our report concerned native cattle of Panama and there is no way to identify the cattle carriers we found in the old herd, we have made another survey and in this series of cattle we know that they were all born and raised in the Republic of Panama. We have selected cattle where the horse disease had not been present for some period of time and other cattle known to be on ranges where the disease was present. In addition, we have inoculated six more calves in order to follow the course of the parasites from a known date of infection.

SURVEY METHOD USED

Large guinea pigs were selected for use and 3 cc. of whole blood from the jugular vein of each steer was injected into the peritoneal cavity. In some instances more than one pig was used. In order to favor the guinea pigs as much as possible we did not take them into the remote cattle ranges since it was possible to identify each owner's shipment of cattle to the Panama City slaughterhouse and inoculate the pigs at the time of the slaughter. A

thick blood film was made from a drop of the blood that was to be injected in the pigs. The total number of cattle handled in this manner was 310 but we can use for study only 234 of this number since none are to be considered in which the guinea pigs failed to live at least two weeks after the inoculation. We selected cattle from three provinces of the Republic. The province of Panama had been negative on blood surveys for the disease for almost two years while the disease was still present, in a low incidence, in the provinces of Coclé and Herrera. The period of time covered was from September, 1932 to August, 1934. The results are shown in table 1.

TABLE 1

PROVINCES	NUMBER OF CATTLE TESTED	NUMBER OF CATTLE POSITIVE
Panama	71	0
Coclé	148	4 (2.7 per cent)
Herrera	15	1 (6.6 per cent)
Total	234	5 (2.1 per cent)

None of the thick-blood-films made from the surveyed cattle at the time of the guinea pig inoculations revealed trypanosomes (*T. hippicum*).

These cattle came from known ranges where there was contact with herds of horses but the ranges were not fenced and, therefore, permitted wide excursions as compared to the fenced ranges of the Canal Zone mentioned in the last report (1). Had this survey been conducted in 1929 to 1931, we believe the cattle carrier index would have been higher. Control measures seem to have greatly reduced the disease among the horses and mules. We can now state with certainty that native raised cattle can act as unharmed reservoirs for this parasite.

The question of how long they may be carriers could not be answered by field studies in a satisfactory manner. We selected six native calves that were old enough to be weaned and kept at our Veterinary Station. These were subjected to guinea pig

inoculation and daily thick film examinations until we were satisfied they were clean animals and then they were given superinfections by injecting into the jugular vein all of the blood we could get from a guinea pig carrying a strain of *T. hippicum*. This provided a known date of infection that would permit a study of the length of the carrier period. When such large quantities of blood are injected into the jugular vein, it is possible in a very few moments to take a drop of blood from the ear and find a few trypanosomes.

This does not last very long for a great many of the parasites seem to be killed. A period of incubation of five to eight days must then pass before blood films are apt to reveal the parasite, and after a few more days it is no longer possible to use this method. The guinea pig (intraperitoneal injections of 3 cc. of whole blood) will continue to give results for months and even after this method fails they respond for some time to the complement fixation test. We have not kept animals long enough to learn the average period of time when this response fails. Experience with horses and mules indicates that it would eventually fail to respond.

CALF RECORDS

Calf number 2

It was given an intrajugular inoculation on March 20, 1933 from guinea pig 55A. The dose used was 4 cc. of whole blood. A thick blood film from this pig averaged about 75 trypanosomes to the field (570 diameter magnification). All blood films before the inoculation were negative for trypanosomes but at one time, 2 spirochetes and a single piroplasm were found. A blood film was taken from the ear of the calf fifteen minutes after it was inoculated and 21 trypanosomes (*T. hippicum*) were found. The blood films of this calf were not taken for a period of two days following its inoculation. The days on which the films were positive following that date are as listed in table 2.

Guinea pig tests. Two pigs were inoculated each week from April 17, 1933 to January 10, 1934. The blood was taken from the jugular vein and 2 cc. of this whole blood was injected into

the peritoneal cavity of each pig. The results are shown in table 3.

TABLE 2

Days on which blood films from calf 2 were positive following inoculation

DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR	DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR
1933		*1933*	
March 22	3	March 29	5
March 23	2	March 30	2
March 26	1	April 1	2
March 27	1	April 2	1
March 28	1	September 29	1

It was killed on February 12, 1934, and was fat enough to be used for baby beef.

TABLE 3

Results of injections of whole blood into peritoneal cavity of guinea pigs

PIG NUMBER	DATE INOCULATED	DATE POSITIVE
	1933	*1933*
86A	April 25	May 6
87A	April 25	May 6
92A	May 3	May 12
93A	May 3	May 12
96A	May 10	May 20
97A	May 10	May 20
109A	May 18	May 31
118A	May 24	June 7
126A	June 2	June 10
127A	June 2	June 16
154A	June 21	June 29
155A	June 21	July 5
171A	July 5	July 17
182A	July 12	July 24
245A	August 17	August 31

We used 94 pigs on this calf from April 17, 1933 to January 10, 1934 and 15 of them became positive. The remaining 79 pigs died within ten days after they were inoculated. From August 17, 1933 to January 10, 1934, all pigs were negative.

Calf number 3

This animal was given an intrajugular inoculation at 3:25 p.m., March 20, 1933, from guinea pig 55A. The dose used was 4 cc. of whole blood. A thick blood film from this animal also averaged about 75 trypanosomes to the field.

This calf had never shown anything in its blood prior to its inoculation except a few piroplasma. A film from its ear fifteen minutes after its inoculation showed 9 *T. hippicum* parasites and 5 piroplasma forms. No films were taken for two days following the inoculation. The days when the blood films were positive after that time are listed in table 4.

TABLE 4

Days on which blood films from calf 3 were positive following inoculation

DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR	DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR
1933		*1933*	
March 22	1	March 28	10
March 24	2	March 29	2
March 26	2	March 30	2
March 27	8	April 1	4

Further films were all negative up to the date it was killed, March 14, 1934.

Guinea pig tests. Two pigs were inoculated each time at weekly intervals. The dose of whole blood from the jugular vein was 2 cc. and this was injected into the peritoneal cavity. The results are shown in table 5.

We used 94 pigs in tests on this calf from April 17, 1933 to January 10, 1934. There were 13 that developed the disease and 81 that were negative or died too soon after inoculation to pass the incubation period.

Calf number 4

This calf was given an intrajugular dose of 10 cc. of whole blood from guinea pig 18B. This was done on February 26, 1934 and a thick blood film of the blood used averaged about 50 trypanosomes to each microscopic field. Fifteen minutes after

the calf was inoculated a blood film was made from its ear that revealed 15 *T. hippicum* parasites. No further blood films were taken for eight days and the results of these are shown in table 6.

TABLE 5

Results of injections of whole blood into peritoneal cavity of guinea pigs

GUINEA PIG NUMBER	DATE INOCULATED	DATE POSITIVE
	1933	*1933*
73A	April 17	April 26
74A	April 17	April 26
83A	April 24	May 5
95A	May 3	May 13
98A	May 10	May 24
99A	May 10	May 24
108A	May 18	May 31
121A	May 24	June 6
94A	June 2	June 12
134A	June 7	June 20
135A	June 21	June 21
173A	July 5	July 24
223A	August 2	August 17

TABLE 6

Results from blood films from calf 4

DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR	DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR
1934		*1934*	
March 6	1	March 13	1
March 7	5	March 14	1
March 8	163	March 15	1
March 9	1	March 16	1

Further blood films were all negative to the date of death, September 7, 1934.

Guinea pig tests. Three pigs were inoculated each time at monthly intervals for five months. The record of these pigs is shown in table 7.

Calf number 5

This animal was given an intrajugular dose of 10 cc. of whole blood from guinea pig 490A. This was done on February 26,

1934. A drop of the blood from the pig at the time contained about 50 trypanosomes to the microscopic field. A drop of blood from the calf's ear fifteen minutes after its inoculation, showed 5 trypanosomes. No further blood films were taken for five days and the results of those films are given in table 8.

TABLE 7

Guinea pig records

DATE	GUINEA PIG NUMBER	RESULT
1934		
March 28	60B	Became positive, April 6
March 28	61B	Became positive, April 12
March 28	62B	Became positive, April 21
April 26	107B	Never positive, died, May 4
April 26	108B	Became positive, May 5
April 26	109B	Became positive, May 8
May 28	165B	Never positive, died, June 18
May 28	166B	Never positive, died, July 9
May 28	167B	Never positive, died, August 3
June 26	246B	Never positive, died, July 30
June 26	247B	Never positive, died, August 17
June 26	248B	Never positive, died, August 14
July 27	293B	Never positive, died, August 27
July 27	294B	Never positive, died, September 1
July 27	295B	Never positive, died, August 6

Guinea pig tests. Three pigs were inoculated each time from this calf at monthly intervals for five months. The results are shown in table 9.

Calf number 6

This calf was given an intrajugular inoculation of 15 cc. of whole blood from guinea pig 6B and at the time a drop of its blood revealed about 80 trypanosomes to each microscopic field. The inoculation was performed on February 26, 1934. A drop of

TABLE 8

Results from blood films from calf 5

DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR	DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR
1934		*1934*	
March 3	1	March 9	4
March 5	2	March 10	3
March 6	2	March 11	3
March 7	4	March 12	1
March 8	39	March 14	1

The films on all other days to the date it was killed, August 30, 1934, were negative.

TABLE 9

Guinea pig records

DATE	GUINEA PIG NUMBER	RESULTS
1934		
March 28	63B	Became positive, April 12
March 28	64B	Became positive, April 14
March 28	65B	Became positive, April 6
April 26	110B	Became positive, May 5
April 26	111B	Became positive, May 5
April 26	112B	Became positive, May 8
May 28	168B	Never positive, died, June 17
May 28	169B	Never positive, died, July 21
May 28	170B	Became positive, June 9
June 26	243B	Never positive, died, August 6
June 26	244B	Never positive, died, July 20
June 26	245B	Never positive, died, July 24
July 27	296B	Never positive, died, August 11
July 27	297B	Never positive, died, September 4
July 27	298B	Never positive, died, September 4

blood taken from the calf's ear fifteen minutes later revealed 5 trypanosomes. No other films were made for five days. Daily film results are shown in table 10.

Guinea pig tests. Three pigs were inoculated each time from this animal at monthly intervals for eight consecutive months. The results are shown in table 11.

Calf number 7

This calf was given an intrajugular inoculation of 12 cc. of whole blood from guinea pig 463A on February 26, 1934. The pig's blood revealed an average of 150 trypanosomes to each microscopic field. A blood film fifteen minutes after inoculation taken from the ear of the calf showed 12 trypanosomes. No

TABLE 10

Results from blood films from calf 6

DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR	DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR
1934		*1934*	
March 3	3	March 14	3
March 9	1	March 16	1
March 11	1		

The blood films were negative on other days to the date it was killed, December 10, 1934.

further films were made for five days and the results of these films are shown in table 12.

Guinea pig tests. Three pigs were inoculated each time from this calf at monthly intervals for five consecutive months. The results are shown in table 13.

COMPLEMENT FIXATION TEST

Specimens of blood were taken from calves 4, 5, 6 and 7 August, 1934 and were sent to the Bureau of Animal Industry where a complement fixation test for trypanosomiasis was applied and all were reported as positive. These specimens on numbers 3, 5, and 7 were taken after they had become negative to blood films and guinea pig tests but number 6 was still positive to the pig inoculations.

At the same time that these sera were sent to Washington we

collected blood serum from 16 horses that represented cured cases of trypanosomiasis (1930 to 1931) that have been in use since

TABLE 11

Guinea pig records

DATE	GUINEA PIG NUMBER	RESULTS
1934		
March 28	66B	Became positive, April 18
March 28	67B	Became positive, April 9
March 28	68B	Became positive, April 16
April 26	113B	Became positive, May 7
April 26	114B	Never positive, died, May 4
April 26	115B	Became positive, May 3
May 28	171B	Became positive, June 13
May 28	172B	Became positive, June 7
May 28	173B	Became positive, June 8
June 26	240B	Became positive, July 9
June 26	241B	Never positive, died, July 31
June 26	242B	Never positive, died, July 13
July 27	299B	Never positive, died, August 16
July 27	300B	Never positive, died, August 6
July 27	301B	Became positive, August 11
August 27	367B	Never positive, died, October 7
August 27	368B	Became positive, September 11
August 27	369B	Never positive, died, September 1
September 26	429B	Never positive, died, November 27
September 26	430B	Never positive, died, November 30
September 26	431B	Never positive, died, October 13
October 26	15C	Never positive, died, November 28
October 26	16C	Never positive, died, December 31
October 26	17C	Never positive, died, December 31

The calf was killed December 10, 1934.

then as saddle animals and brood mares. These all gave a negative response to the complement fixation test and it is believed

TABLE 12

Results from blood films from calf 7

DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR	DATE	NUMBER OF TRYPANOSOMES POSITIVE FOR
1934		*1934*	
March 6	1	March 10	1
March 7	3	March 12	1
March 8	36	March 13	2
March 9	2	March 14	1

No other films were found positive up to the date of its killing which occurred August 28, 1934.

TABLE 13

Guinea pig results

DATE	GUINEA PIG NUMBER	RESULTS
1934		
March 28	69B	Never positive, died, April 2
March 28	70B	Became positive, April 5
March 28	71B	Never positive, died, April 21
April 26	116B	Became positive, May 3
April 26	117B	Became positive, May 3
April 26	118B	Became positive, May 7
May 28	174B	Never positive, died, July 9
May 28	175B	Never positive, died, July 19
May 28	176B	Never positive, died, June 15
June 26	237B	Never positive, died, July 21
June 26	238B	Never positive, died, August 3
June 26	239B	Never positive, died, August 10
July 27	302B	Never positive, died, August 21
July 27	303B	Never positive, died, September 17
July 27	304B	Never positive, died, August 27

All six of these calves grew as rapidly as any range animals and they had more fat than the average animal one sees killed for beef. These were killed and used as beef by the laboratory and veterinary personnel.

that the calves would eventually have given a negative response. No doubt the application of this test to a herd of cattle would reveal a greater carrier index than the method we used but our interest centers about the cattle carrier that is able to infect vampire bats.

Cattle strains of Trypanosoma hippicum

The five strains recovered from range cattle were all used on healthy horses. Acute attacks of trypanosomiasis resulted, the parasites became very abundant and all the horses responded to the complement fixation test.

Vampire bat feedings on cattle carriers

In another article (2) we discussed the probability of the vampire bat, *Desmodus rotundus murinus*, being able to acquire the disease by feeding on cattle carriers. Dunn (L. H.) has been able to experiment with three of these bats on some of the calves considered in this article. Further studies of this nature are desired but we wish to record here the progress with this experiment. He liberated two male vampire bats (nos. 33 and 34) in the screened stall with calf 2 on March 28, 1933. Bat 33 remained with this calf until February 7, 1934 when it was brought back to the Gorgas Memorial Laboratory. Bat 34, on April 22, 1933, was removed from the stall of calf 2 and put in the stall with calf 3 until August 12, 1933 when it was again placed in the stall with calf 2 until February 7, 1934. These bats had no other access to food than the blood meals they took from the calves and a shallow dish of water. These bats fed on these calves a total of 315 nights. Bat 33 was with calf 2 on six nights when the blood films of the calf contained a few trypanosomes and guinea pig inoculations all proved positive until August 17, 1933. Bat 34 did not feed on calf 3 at any time when its blood films were positive for trypanosomes but the guinea pig inoculations from the calf all proved positive during this time the bat was feeding on it. Bats 33 and 34 never acquired the disease. Bat 31 was placed in the stall with calf 3 on March 28, 1933 and on this date the calf revealed 10 trypanosomes in its blood film

and continued to show a very few for the next two days and again on April 1. Guinea pigs inoculated from the calf during the stay of the bat also developed the disease. We dare not handle these bats too much so daily blood films were not made from it but its blood was negative on April 5. The next film taken was on April 13, and this was positive for *T. hippicum*. There was an average of 2 trypanosomes to the field (570 diameters). The bat was brought back to the laboratory on April 15. It fed on guinea pig 75A on April 17 and the pig's blood film was found positive on May 2. No blood films were taken on the guinea pig between the time of the bat feeding and May 2, so we can not report its first appearance in the blood. Bat 31 died on April 19, 1933. The oral secretion secured by gently applying a wire loop to the membrane was prepared in films that could be stained and these films revealed 28 *T. hippicum* parasites in the saliva. The experiment ended with but one of the three bats acquiring the diseases from a light cattle carrier and this occurred at a time when the blood films were positive for a few trypanosomes. It would appear that these bats require a fair number of trypanosomes in the peripheral blood if they are to acquire the disease in any high incidence. Undoubtedly the alimentary tract of the bat succeeds in killing off many of the ingested parasites because guinea pigs receiving 2 to 3 cc. of blood from the calves into their peritoneal cavities did acquire the disease during intervals when it can be said that the bats each averaged 16 cc. of blood every night at their feedings. Considering the vast number of bats that regularly attack cattle, horses and mules it, nevertheless, is possible for an occasional bat to acquire the disease from a cattle carrier.

SUMMARY

1. Native cattle of the Republic of Panama can act as an important reservoir for *T. hippicum*, the cause of equine trypanosomiasis. Our survey methods indicate that the cattle carrier index will range from 2 to 6 per cent where the horse disease is present.

2. *Length of the carrier state.* Thick blood films will reveal a scant number of trypanosomes from the time of inoculation for

about two weeks. The six calves ranged from eleven to eighteen days. One film from calf 2 was positive on a day about six months after its inoculation but there were none positive between the twelfth day and that remote period.

Guinea pig tests show that the six calves failed to infect anywhere from two to six months after they acquired the infection. The complement fixation test applied to four of the calves showed a positive response at the end of six months in three and at the end of nine months in one. The calves were killed and we can not state with certainty when this response might have failed. We know it failed to respond in 16 horses cured of the disease in 1930 to 1931 and tested in August, 1934.

3. The complement fixation test should be of practical use in surveying a herd of cattle for carriers.

4. *Physical condition of the calves.* The calves grew in a normal manner and were fatter than cattle of a similar age left on the range.

5. The 5 strains of *T. hippicum* recovered from range cattle were injected into healthy horses. An acute trypanosomiasis developed similar in all respects to the strains formerly recovered from horses.

6. Three vampire bats (*Desmodus rotundus murinus*) were fed on cattle carriers. One acquired the disease and transferred it to a guinea pig. Two failed to acquire the disease. It is quite evident that the peripheral blood of an animal must contain a fair number of the trypanosomes at the time the bat feeds on it.

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