STUDIES ON FLAGELLATES OF THE BATS *Tadarida brasiliensis* AND *Myotis velifer* AND OF THEIR ECTOPARASITES IN OKLAHOMA

By

AUGUSTO ALBERTO CADENA

Bachelor of Science

Universidad de los Andes

Bogota, Colombia

1964

Submitted to the faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
July, 1967

STUDIES ON FLAGELLATES OF THE BATS Tadarida brasiliensis AND Myotis velifer AND OF THEIR ECTOPARASITES IN OKLAHOMA

Bryant P. Glass
Thesis Adviser

E. W. Rosch

Ray W. Jones

N. D. Durham
Dean of the Graduate College

PREFACE

The objectives of the present study of the flagellates on the bats Tadarida brasiliensis and Myotis velifer and on their ectoparasites were to: (1) find Trypanosoma cruzi or T. cruzi-like in bats and its relationship in regard to age, sex, and season; (2) attempt to find a possible vector in their ectoparasites.

Dr. Bryan P. Glass, major adviser, and Drs. R. W. Jones, and Everett Besch served on the advisory committee and criticized the manuscript. George Rogers and Stanley Rouk helped to make field collections. Drs. C. J. Marinkelle from the Andes University in Bogota, and Franklin G. Wallace from the University of Minnesota helped in the identification of the flagellates. The assistance of all these people and that of N. R. Cooley for his data is appreciated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

In all the Americas from Argentina to the United States, bats naturally infected with trypanosomes have been found and numerous publications have been written concerning the incidence of infection and geographic distribution (Wood, 1952; Kagan et al., 1966; Marinkelle, 1966). Bats are not the only animals infected with trypanosomes. Many other wild mammals are also known to harbor hemoflagellates, and more than 100 species have been described as harboring trypanosomes similar to T. cruzi (Marinkelle, 1966). North American investigators, especially in the United States, have emphasized wild mammals rather than humans as the source of Trypanosoma cruzi or T. cruzi-like organisms, because very few human cases of trypanosomiasis have been reported in their study area (Anonymous, 1956; Woody and Woody, 1955).

In South America about seven million people are infected with Chagas' disease or American trypanosomiasis (WHO Technical Report, 1960), and investigations in wild mammals have been relegated to a secondary place. However, this type of study is increasingly attracting the attention of workers who are interested in finding the source of trypanosome infection in humans. As a product of this interest in Colombia, many species of wild mammals, bats, monkeys, opossums, and armadillos have been found to harbor flagellates (Marinkelle, 1966). A number of investigators in other countries have found many genera of

bats infected with trypanosomes, of which only the genera Myotis and Tadarida have significance for the present study (Table I). There have been both positive and negative results in investigations concerned with these two genera (Table II).

Compared with South America, North American studies of trypanosomes in wild mammals are more extensive, and a great deal of investigation has been done in all of the southern states, especially those that adjoin Mexico. Recently in several states of the southeastern United States surveys have been made of the trypanosomes of wild animals. The difference in the host species is great between the eastern and western states, and it is important to identify all potential hosts whose trypanosomes are capable of infecting man.

In the United States the infection of wild animals has been reported in ten states (Kagan et al., 1966; Bice, 1966; Brooke, 1957; Herman, 1962; McKeever, 1958; Walton, 1956). In the western states, the species infected with trypanosomes are small mammals, bats, rats, squirrels, ground squirrels and wild mice (Wood, 1952). In the East trypanosome infection is in medium-sized wild mammals, opossums, Didelphis marsupialis, skunks, Mephitis mephitis, raccoons, Procyon lotor, and grey foxes, Urocyon cinereoargenteus. Although in recent years several workers have been conducting surveys of wild mammals in different parts of the country, they are presently emphasizing medium-sized mammals rather than small ones (Kagan, et al., 1966).

Apparently investigations are biased toward medium-sized mammals because of the distribution of the triatomid vector of the trypanosomes. The problem is to determine the route of infection of wild mammals. The species of vectors are not the same in both South and North America,

TABLE I

BATS OF GENUS MYOTIS FOUND INFECTED WITH TRYPANOSOMES

Species	Country	Author and Year	No. Pos./No. Neg.
M. nigricans	Argentina	Romana, 1936	?
M. nigricans	Colombia	Marinkelle, 1966	2/4
M. nigricans	Venezuela	Pifano, 1939	?
M. dinelli	Argentina	Mazza, 1940	?
M. yumanensis	United States (Calif.)	Mitchell, 1956	2/75
M. yumanensis	United States (Ariz.)	Wood, 1949	9/?
M. ocultus	United States (Calif.)	Wood, 1952	5/?
M. velifer	United States (Ariz.)	Wood, 1952	1/?
M. velifer	United States (Ariz.)	Wood, 1943	3/?

TABLE II

BATS OF GENERA MYOTIS AND TADARIDA FOUND NOT INFECTED WITH TRYPANOSOMES

Species	Country	Author and Year	No. Pos./No. Neg.
M. nigricans	Brazil	Dias, 1940	2
M. thysanoides	United States (Calif.)	Davis, 1952	8
M. californicus	United States (Calif.)	Mitchell, 1956	6
T. mexicana	United States (Calif.)	Wood and Wood, 1937	1
T. mexicana	United States (Calif.)	Mitchell, 1956	28*
T. mexicana	United States (Calif.)	Davis, 1952	37
T. brasiliensis	United States (Texas)	Lesser, 1959	196

*Personal communication of Wood to Mitchell. They examined numerous Tadarida that were negative.

although investigators are agreed that in both Americas the principal vectors are triatomids, rather than any other haematophagous insect. This observation is particularly pertinent in the epidemiology of disease transmission to humans, because only insects defecating during or immediately after feeding can be efficient vectors for Chagas' disease (Kagan, et al., 1966). However, there are many other vectors that could transmit the flagellates among wild mammals, and it has been stated above that many wild mammals have been found infected in North America. Even though there are many species of trypanosomes in animals, this does not necessarily mean that all of the trypanosomes found in animals are able to infect humans or that their introduction could produce Chagas' disease. Some of them are morphologically similar to T. cruzi, and are called "T. cruzi-like." It is also significant that the reported infection rate in triatomids in the United States is 20%, a rate very similar to the 20-30% reported for endemic areas of Chagas' disease in South America (Yaeger, 1960).

The principal vector of T. cruzi in bats in South America is a triatomid, Cavernicola pilosa. This triatomid in only found in the tropic of certain Latin-American countries (Marinkelle, 1967). Although the role of ectoparasites and the transmission of bat flagellates is not well understood, Van Den Berghe, (1963) mentioned that in Africa a bug (Stricticimex brevispinosus) is responsible for the transmission of the so-called broad-type trypanosomes among the insectivorous bat Hipposideros caffer, and also established that the trypanosomes found in chiroptera are transmitted in the same way as the T. lewisi group defined by Hoare (Van Den Berghe, 1963).

It is apparent that among the small mammals bats have been under-

estimated as a possible reservoir of trypanosomal infections. Very little work has been done on them in recent years, especially in those states in the United States where the infection has not been detected. The objectives of this study were to demonstrate the incidence of trypanosomal infection in bats in Oklahoma, although it is not one of the states reported to have wild mammals infected with these flagellates (Trager, 1958), and to determine the potential vector of the flagellates among the ectoparasites found in the bats.

CHAPTER II

MATERIALS AND METHODS

Two species of bats were collected; Tadarida brasiliensis from June, 1966 to early September, 1966, and Myotis velifer from December, 1966 to early April, 1967. Both species were collected in the same area called Vickery Caves, located in the west-central part of Oklahoma in Major County (Glass, 1959). The bats, kept in cages containing water, were left in a refrigerator at an average temperature of 4^{o} C.

All specimens, and ectoparasites collected from both species, and also two triatomids, Triatoma sanguisuga, found near the cave, were examined in the laboratory for the presence of flagellates.

During the summer and autumn Tadarida brasiliensis were collected and separated by sex and age. Because this species is migratory, no specimens were collected during the winter. The other species, a hibernating type of bat, M. velifer, was not collected during the summer. No newborn or young were examined.

Both species of bats were heavily infested with several kinds of ectoparasites, mites, ticks, fleas, and bat flies. All types were examined for the presence of flagellates. Both bats and ectoparasites were examined according to different methods during the course of the experiment. A review of these techniques follows.

Blood obtained by heart puncture was examined under a microscope, one or two drops were put on a slide and covered with a coverslip.

Examination, using a binocular microscope with 10x oculars, was made at 43x, high dry objective, and on a very few occasions at 97x, immersion oil objective. Each slide was examined for at least five minutes, using different fields at random. The best results were obtained by this method. These data compare with those obtained by Davis (1952), who called it a fresh preparation.

Another method used was the centrifugation technique developed by Deane and Kirchner (1962). The so-called distilled-water method for centrifugation consists of the mixing of one part of blood plus five parts of distilled water, and the addition within three to five seconds of five parts of 1.6 gram NaCl in 1,000 ml. water. This is centrifuged for 20-30 minutes at 2,000 to 3,000 r.p.m. and the deposit is examined. This method was only used on a few occasions, because the same results were obtained as with fresh preparations, and it was only advantageous when many organisms were needed for a cultivation. The method of Hoare (1964) was not used although many workers believe that it is one of the best methods for the isolation of flagellates (Olsen, 1964,65).

Permanent preparations were made by extension smears using two methods, thin and thick smears. Thin smears were made by the general technique used in any work of haematology. Thick drop preparations were done on certain occasions using the technique described by Marinkelle (1967). This technique allows for the deformation of trypanosomes. Smears made by this method must be thin enough that the numbers of a watch or letters of a newspaper may easily be seen through it, and the slide preparation is allowed to dry from 5-12 hours. If the room humidity were more than 50-60%, slides should be dried in a dissicator. If the laboratory temperature were below 20^{o} C, the slide

should be placed in an incubator for one to two hours. After complete dessication, the preparation was covered with methylene blue solution A (Table III) for 5-20 seconds, then was dipped in solution B (Table III) until all haemoglobin was removed. After that the slide was placed on end to dry.

The permanent preparations were fixed according to two methods; dipping the slides in methanol for three to four minutes, or using fumes of osmic acid for three to four minutes (Taliaferro, 1923).

The period of coloration of the fixed slides was 1-24 hours in a solution using two drops of commercial Giemsa stain per each ml. of solution B (Table III). The stained slides were washed in tap water and dried, then observed under a microscope using the high dry objective. When a trypanosome was found it was observed under the immersion oil objective for better appreciation of the flagellates.

Macerated ectoparasites and blood samples were cultured in a diphasic medium with a solid base composed of a modified NNN medium of Novy, MacNeal, Nicolle (Tobie, 1964) and a fluid overlay (Locke's solution). T. cruzi could survive for as long as 5 years in this medium (Packchanian, 1943).

TABLE III

SOLUTIONS FOR THICK DROP PREPARATIONS

Solution A		Solution B	
Methylen blue powder	1 gm.	Na_2HPO_4 (anhydrous)	6 gm.
Na_2HPO_4	3 gm.	KH_2PO_4	5 gm.
KH_2PO_4	1 gm.	Distilled water	1000 ml.
Distilled water	1500 ml.		

The composition and method of preparation of the NNN medium is: Agar 14 gm., NaCl 6 gm., and distilled water 900 ml. The water is brought to boiling and the salt and agar are dissolved in it. The medium is distributed in test tubes to about one-third capacity. The caps of the test tubes are screwed on, and they are sterilized in the autoclave for 20 minutes at 132° C with 15 lbs. of pressure. Tubes containing this agar base may be stored in the refrigerator and used as needed. In order to use them, the tubes are placed into hot water to melt the agar. Then they are cooled to 48° C to 50° C. To each tube approximately one-third as much sterile whole human blood as the volume of agar is added. The blood and agar are thoroughly mixed by rapid rotation of the tubes and then placed each in a slanting position on ice or in a refrigerator. After the tubes are cool, they are placed in an upright position and incubated for 24 hours at 36° C to determine sterility.

The fluid overlay of Locke's solution is composed of NaCl 8.0 gm., KCl 0.2 gm., $CaCl_2$ 0.2 gm., KH_2PO_4 0.3 gm., Glucose 2.5 gm., and distilled water 1,000 ml. The components are sterilized in the autoclave, in the same manner indicated above, then about 2 ml. of liquid overlay is placed in each tube (Levine, 1961). In this work 250 units per ml. of penicillin-streptomycin mixture were added to the Locke's solution for each ml. of media. The tubes with the diphasic medium were inoculated with blood obtained from bats and cultured at 22° C to 23° C. The utmost attention to asepsis in securing and inoculating the material to be cultured is essential.

When trypanosomes were found in the bats, samples of blood were inoculated directly intraperitoneally in mice of two weeks of age and

then after one week the blood of the mice was examined for the presence of flagellates. This was accomplished by squeezing out a drop of blood from the tail on a slide which is then covered with a coverslip.

Impression smears were made by pressing different organs on a slide, taking care that the organs were not bloody, and then were stained with Giemsa stain.

Measurements of the trypanosomes were made using enlarged photographs taken from the stained slides, and a stage micrometer was used to determine the size of the enlargement.

CHAPTER III

RESULTS AND DISCUSSION

The literature contains no references to Oklahoma as a source of animals that harbor trypanosomes. In a survey of certain wild rodents of Oklahoma (*Neotoma* ssp., *Sigmodon* sp., and *Peromyscus* sp.) and three triatomids found in the nests of three rats, negative results were obtained for trypanosomes (Trager, 1958). However Cooley (unpublished data) in 1956 found trypanosomes morphologically similar to *Trypanosoma cruzi* in *Myotis grisescens* (1 of 27 individuals) and in *Myotis velifer* (10 of 30 individuals) during a period of one and a half years, but identification of this trypanosome was not proved by any means other than stained blood smears. The sources of the bats were Adair Bat Cave (*M. grisecens*), Alabaster Caverns (*M. velifer*), Reed Cave (*M. velifer*), and Anderson Creek Caves (*M. velifer*). The location of these caves in order is in Adair, Woodward, Woods, Greer and Harmon Counties (Glass, 1959). Later in this work, the distribution of infected bats found in this work is compared with those found by Cooley (Figure 1).

In the present study 321 *Tadarida brasiliensis* were examined and found negative in regard to flagellates of any kind, either in fresh preparation, stained smears, impression slides, or cultivation in NNN cultures (Table IV).

The bats were separated by age, sex, and season. The three age categories were; newborn without hair and usually attached to their

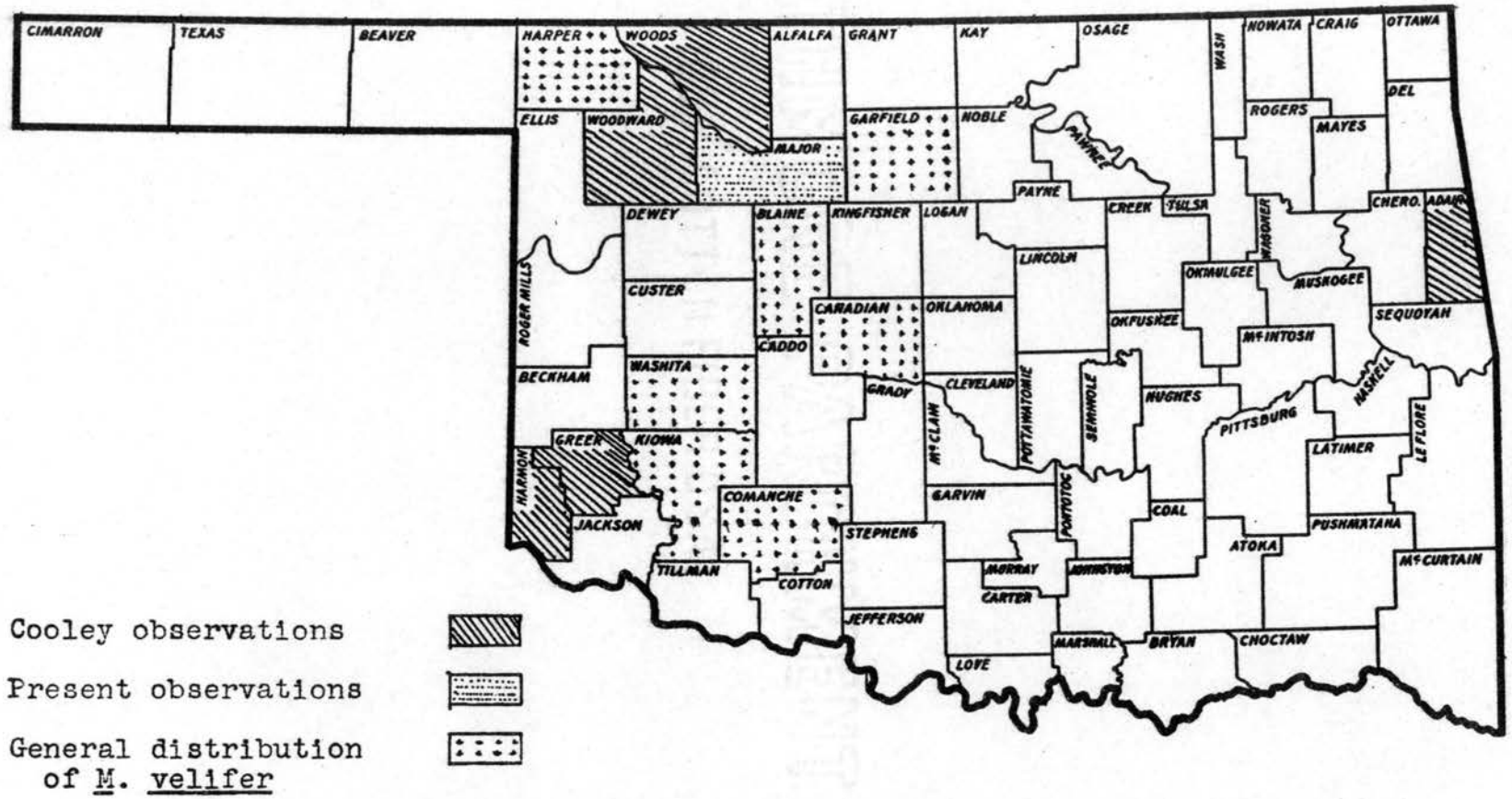

Figure 1. Distribution by Counties of Myotis ssp. Found Infected With Trypanosomes

TABLE IV

OKLAHOMA BATS EXAMINED FOR TRYPANOSOMES
(Figures in parenthesis indicated infected animals)

		Myotis velifer	*Tadarida brasiliensis*
Spring	March	23 (3)	..
	April	8 (2)	..
	May	..	..
Summer	June	..	85
	July	..	105
	Aug.	..	99
Fall	Sept.	..	32
	Oct.	..	..
	Nov.	..	..
Winter	Dec.	29 (3)	..
	Jan.	33 (5)	..
	Feb.	35 (7)	..
Total Exam.		128	321
Total Infect.		20	0
Per cent of Infect.		15.6	0

mothers, juveniles with gray pelage, and adults with brown pelage. The number of specimens collected consisted of ten specimens of newborn, constituting 3.1%, 42 juveniles of 13%, and 269 adults or 83.9% of the total number. The adults represented two types; one, adults which were recently returned from migration, collected in early summer, and second, the juveniles which developed the adult pelage during the summer and were collected in the early fall. In this study the separation by age was done assuming that before adulthood they could become infected and then lose the infection with time due to development of resistance, as has been observed in other types of trypanosomes, for examples T. lewisi in rats (Taliaferro, 1924). All the results of this investigation of Tadarida were negative, and trypanosomes were not observed to be present at any age.

The results were negative also when the differentiation was made by season, although collecting was done only during the time the bats were in Oklahoma between June and September. However, this does not eliminate the possibility that they could become infected in the places to which they migrate, and then when they return to Oklahoma lose the infection due to unknown factors. The same negative results were obtained in some investigations done with the genus Tadarida in other states (Table II), and in Oklahoma, Cooley (1967) examined 46 Tadarida mexicana with results similar to those discussed above. In this study it was not possible to determine if the sex of Tadarida had any implications in regard to trypanosomal infection, for of the 174 females and 147 males collected all were negative.

On the other hand, fresh preparations of blood from 128 Myotis velifer were examined, and a total of 20 (15.6%) were found infected

with trypanosomes (Table IV). Flagellates were encountered very quickly in all positive preparations, involving the scanning of only four or five fields to find them, usually with two or three trypanosomes per field. This means that infection was very heavy, except for two bats in which about 50 fields had to be examined to find flagellates. These two had very few trypanosomes on the entire slide. For the purpose of culture and staining it was necessary to use the centrifugation method in those bats with few trypanosomes.

The difference in infection according to age and season was not determined because no specimens were obtained during the summer (Table IV), and no newborn or juveniles were captured during this study. Neither were differences found in this study in regard to sex (Table V). Both males and females were found infected with a ratio of 1:1. Further study might indicate a difference in juveniles and newborn, but according to the results in adults in this work, it seems that there are no differences related to sex. The presence of trypanosomes in juveniles and newborn could provide information about the life cycle of trypanosomes in bats, determine the age of infection, and indicate whether the newborn or juveniles are more susceptible to infection than adults.

TABLE V

SEX RATIO OF INFECTED MYOTIS VELIFER

Dec.		Jan.		Feb.		March		April		Total m.	Total f.	Ratio
m.	f.	m.	f.	m.	f.	m.	f.	m.	f.	Infec.	Infec.	
2	1	1	4	6	1	1	2	0	2	10	10	1:1

The stained preparations of trypanosomes found in Myotis velifer seem to be morphologically similar to Trypanosoma cruzi. The trypanosome itself is a curved, stumpy organism with a tapered posterior end. Some individuals are broad and others narrow. The nucleus is rather anterior in position while the kinetoplast is relatively large, ovoid in shape and very close to the anterior end. The undulating membrane is narrow and only slightly convoluted. The flagellum is long, but sometimes due to staining factors it is not clearly visible (Figure 2). The majority of the narrow or slender individuals have a different morphology in relation to the nucleus-kinetoplast complexes which appear to have a rounded kinetoplast and an elongated or bacilliform nucleus. This description is similar to the so-called metacyclic forms found in invertebrate vectors and in tissue cultures at low temperatures (Trejos, 1963; Deane, 1963).

The measurements of the trypanosomes found in the bats are summarized in Table VI. They appear to have a total length of 16.67 microns, which is within the range (length, 16-20 microns) of Trypanosoma cruzi (Levine, 1961). Other measurements in this study seem to be correlated with those of Trypanosoma cruzi found by Dias (1941) in a South American bat, with the exception of the position of the nucleus as indicated by the nuclear index or PN/PA. The trypanosomes found in this investigation have the nucleus very close to the anterior part of the body with a nucleus index of 2.43 which Dias (1941) considered enough evidence to put the trypanosomes in a different genus, but in later works (Trejos, 1963; Deane, 1963; Marinkelle, 1967) this movement of the nucleus in T. cruzi appeared to be affected by the temperature of the host and the vectors, and was not considered a taxonomic characteristic. The hypo-

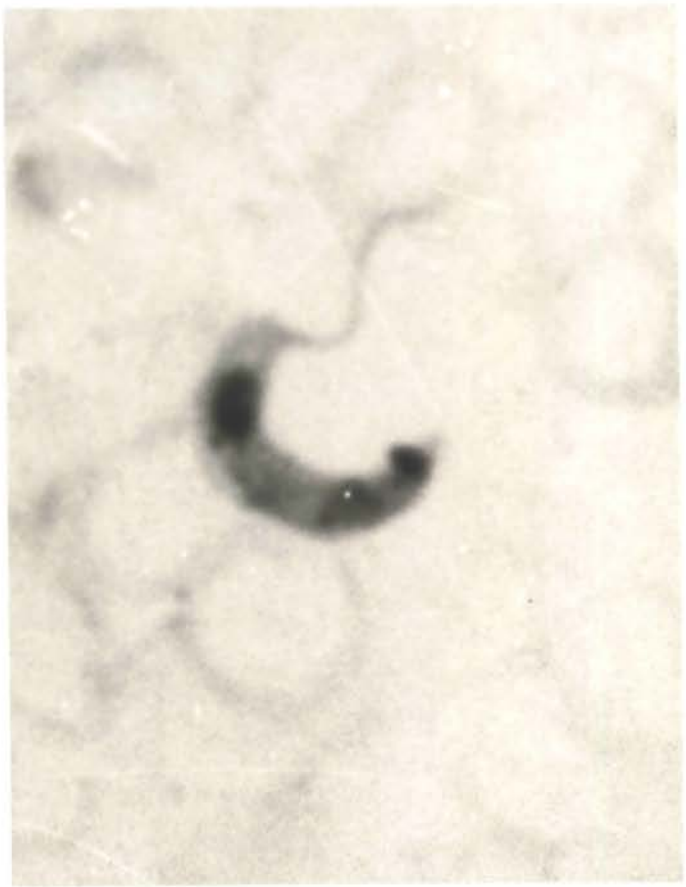

Figure 2. "Trypanosoma cruzi-like" Organisms Found in Myotis velifer.

thesis of Trejos (1963) that temperature is the factor determining the morphology of the parasite, and that the production of slender forms in mammals by putting them in hypothermia seems to be substantiated in this work, in which trypanosomes of hibernating bats have a morphology (indicated here by the nucleus-kinetoplast complexes and nuclear position) distinct from those trypanosomes found in other bats of South America which are not hibernating mammals. The hibernating bats have a temperature very close to the environment of the caves, reaching sometimes 0° C (Twente, 1955).

TABLE VI

MENSURAL DATA OF FLAGELLATES FOUND IN MYOTIS VELIFER
(based on measurements of 100 individuals)

	Mean	Max.	Min.
Total length*	16.67	18.8	14.6
P-K	0.55	0.9	0.3
K-MN	7.44	8.2	6.0
MN-A	3.15	4.4	2.3
Flagellum	5.51	7.7	2.5
PN/NA	2.43	3.2	1.7
Nuclear length	1.94	2.3	1.4
Nuclear width	1.28	1.7	0.8
Body width	1.53	1.9	1.2
Kinetoplast length	0.75	1.5	0.4
Kinetoplast width	1.13	1.5	0.8

*All measurements are in microns

The identification of cultured forms was impossible due to the lack of asepsis and proper equipment, and the cultures became contaminated with fungus and bacteria. Although in fresh preparations it was observed that the flagellates cultured appeared to be in the leptomonad stage, in the stained preparations this was impossible to observe because many bacteria were present which impeded the observation of the organism, and no apparent flagellates were distinguished. The appearance of the leptomonad stage in fresh preparations of cultures was observed four days after inoculation, and the same cultures were negative for all flagellates after 11 days. No further development was observed during these 11 days.

The presence of flagellates in the blood stream of inoculated laboratory mice was not apparent. During this study laboratory mice, strain CD-1, were infected with the trypanosomes found in the bats and observed during a period of two months. The first two weeks they were examined every day and after that period once every week, but no positive results were observed. One factor that could have affected the negative results could be the strain of mice used in this experiment (Bagby, 1962). Usually the strains used for trypanosomes are "C3H", "CFW", or Swiss strain treated with cortisone, (0.2 mg. injected each day intramuscularly for ten days) (Marinkelle, 1966; Norman et al., 1959) but none of these strains were available.

The examination of the impressions of organs of bats infected with trypanosomes were negative for the presence of any stage of development of flagellates. These results and the inoculation of mice indicate that the trypanosomes in this study probably belong to the group called "Trypanosoma cruzi-like". According to the definition given by

Marinkelle (1966) a Trypanosoma cruzi-like is restricted to those trypanosomes morphologically similar to T. cruzi but not capable of producing leishmania bodies in living cells, organs of inoculated laboratory animals, or in tissue cultures.

The geographic distribution of the infected bats in Oklahoma including the results of Cooley (1967) and the present work is the area around Major County in which this study was carried out. As indicated on the map (Figure 1), it can be noted that the locations where infected bats were found are not too far apart, and that there are two main centers of infection. Cooley also examined bats in Adair County, but they were of a different species (Myotis grisescens). Looking at the distribution of Myotis velifer by counties (Glass, 1959) in comparison with the distribution of the Myotis velifer found infected with flagellates, it seems possible that all the populations of M. velifer in the counties from the Southwest to the Northwest are infected. Although there are no records of observations of trypanosomes in bats in the counties between the two major infected groups, the possibility of infection is great due to the fact that the distribution of M. velifer is continuous from the southwest to the northwest part of Oklahoma.

The ectoparasites found in both species of bats were more or less the same families, but the number and species were different. A great number of bat fleas, Sternopsylla texana, were found with Tadarida brasiliensis, with almost every bat being infested. The number of fleas varied from 2-15 on each bat. Another type of ectoparasite found in this particular species of bat was mites. The number of mites present in each bat varied from 15-50, and almost all specimens were infested with them. Identification of this mite has not yet been

obtained. Also bat-flies (Family Streblidae) were present in these mammals. Their number was the least in comparison with the other ectoparasites, but one was observed on almost every Tadarida brasiliensis. The examination, by methods already established, of the ectoparasites mentioned above was negative both in fresh preparation and in culture for the presence of any type of flagellate.

One of the ectoparasites found in Myotis velifer was a bat flea, Myodopsylla gentilis, which was found on only a few occasions (21 specimens), six of them were found to be infected with insect flagellates. The morphology and classification of the flagellates are discussed later.

On M. velifer 58 bat flies, Streblidae, were found. One was observed to have a trypanosome in the blood content of the stomach of the insect, probably due to a recent meal of blood from an infected bat. Mites and ticks were also present, 14 ticks in all and numerous mites (1-15) on each bat. These bats were collected during the winter, and the numbers might be very different in the summer. Also, fleas and streblids have been observed in the sack in which the bats were transported, so the numbers present in the colony could vary from the numbers given above. Since the ectoparasites could pass from one bat to another, the numbers on each bat undoubtedly vary from time to time. The ectoparasites mentioned above were negative in regard to the harboring of flagellates either in fresh preparations, culture, or stained slides.

From the six fleas infected with flagellates observed in fresh preparation and in stained slides, it is concluded that the organisms present were not related to the trypanosomes found in the bats because their morphology was different from that of the bat trypanosomes or any

observed stage of those trypanosomes. This agrees with the conclusion of Wallace (1967) who described it as leptomonas type of promastigate type according to the new terminology of Wallace and Hoare (1966).

The morphology of the flagellates found in the fleas was of two types. The leptomonad form had an elongated body, small, round kinetoplast in the anterior part of the organism close to the nucleus, a rounded or oval nucleus, centrally located, and relatively long flagellum, sometimes not easily distinguishable. It had no undulating membrane; this is a key character for Leptomonas ssp. (Clark, 1959). The tendency of this organism to cluster together in a "rosette" was observed also, as described by Wenyon (1926). As is typical, the general appearance of the crithidial form was rounded with more or less the same characteristics described by Levine (1961). In addition numerous stages between crithidia and leptomona were observed.

Leptomonas have been recorded from at least 11 species of fleas, and five names (L. ctenocephali, L. ctenocephali chattoni, L. ctenophthalmi, L. pattoni, and L. pulicis) are recognized (Wallace, 1966). There are no important differences between the descriptions of these various species. The average measurements of the Leptomonas sp. found in this work (9.3 microns body length, not including the round forms) are within the range of all of the described species. Wallace (1967) stated that it was impossible at that time to make a specific identification of a flea Leptomonas. Until the forms from various hosts have been compared by biological as well as morphological criteria with L. ctenocephali from the dog flea, it is impossible to say whether or not some or all of the flea Leptomonas are a single species. According to Wallace (loc. cit.), Myodopsylla gentilis constitutes a new host record for Leptomonas spp.

A close relationship was also observed between the bats infected with Trypanosomas and the fleas infected with Leptomonas spp. Five of the six fleas infected were collected on bats infected with trypanosomes, but there is no explanation for this relationship. Probably there are more infected fleas in the cave which are not on infected bats, but this needs further study.

Besides the ectoparasites of the bats, two triatomids (Triatoma sanguisuga) were examined by xenodiagnosis for the presence of flagellates, but no organisms were observed. Trager (1958) also examined three of the same triatomids with the same results. It is imperative to make further studies about these insects in Oklahoma because it is well known (Kagan, et al., 1966; Sullivan, 1949) that triatomids infected with trypanosomes have been reported in Texas, and because it is important for the epidemiology to know the distribution of the triatomids infected with trypanosomes, and probably flagellates in triatomids will be found in Oklahoma.

As a result of this study of flagellates, Tadarida brasiliensis and Myotis velifer, which were collected in the same area but in different caves separated by less than half a mile, gave results which were negative for T. brasiliensis, but positive for M. velifer. There are no clear reasons for the differences in infection and our hypothesis is that there is an immunological resistance in Tadarida against the particular strain found in Myotis. Kagan (1962) has demonstrated that mice acquire an immunologic resistance when they are infected with a pathogenic strain, and when reinoculated with immune serum from mice infected with an avirulent strain of T. cruzi. This means that Tadarida could be infected with one particular strain and acquire immunological

resistance which does not permit the persistence of flagellates in the blood stream.

The method of transmission of trypanosomes in bats is virtually unknown. Some scientists have reported several vectors for trypanosomes in bats among mites (L. ponyssus arcautus and Leiognathus lacerani) and bugs (Cimex pipistrelli) (Wenyon, 1926). Wallace (1966) mentioned that mosquitos feeding on bats have been found infected with Blastocrithidia sp. and also mentioned that several species of bat flies (family Nycteribiidae) have been described as harboring Blastocrithia spp. In addition in this work it was mentioned that fleas from bats were infected with Leptomonas sp., so there are a great number of haemotophagous ectoparasites which are potential vectors for flagellates in bats. Because as some authors point out (Van den Berghe, 1963; Marinkelle, 1966; Hoare, 1964) transmission must occur by contamination as in the case of T. lewisi (fleas), T. cruzi (bugs), and T. gravi (flies). With a contaminative type of infection, it is highly probable that all trypanosomes known from Chiroptera are transmitted in the same way which is classical for the trypanosomes belonging to the Stercoraria group of Hoare (1964). All of the insects examined in this study could transmit flagellates through metacyclic forms in feces. It is known that Trypanosoma cruzi is transmitted almost exclusively by triatomids and in Oklahoma triatomids have not been reported to harbor T. cruzi. However, no extensive work has been done on triatomids in this state. It is possible that bats could acquire an infection either by feeding on infected triatomids or by the bites of infected triatomids found in the entrances of the caves.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The reported cases of American trypanosomiasis in some mammals in the United States (Kagan, et al., 1966) stimulated workers to find out more about the geographic distribution and pathogenicity of Chagas' disease in this country. Knowing that Trypanosoma cruzi has been reported in bats from other tropical and subtropical countries, an investigation of two types of bats was carried out, the first one a migratory bat, Tadarida brasiliensis, which goes back and forth to tropical or subtropical regions, and the second one a hibernating bat, Myotis velifer, which spends most of its time in the same area all year around. Collections of both species in Major County were carried out during the period of June, 1966 to April, 1967. A total of 321 Tadarida brasiliensis and 128 Myotis velifer were collected and examined in the laboratory for the presence of flagellates in the blood of the bats or in their ectoparasites. A summary of the results of this study is given below.

1. No flagellates of any kind were present in 321 Tadarida brasiliensis examined, using all types of available methods of investigation.
2. Myotis velifer were found infected with trypanosomes in 15.6 per cent of the 128 bats collected.
3. Identification of the trypanosomes was made by several methods: fresh preparations for the presence of flagellates in bats, stained

smears for the morphology and measurements of the trypanosomes, cultivation for the morphology and study of intermediate stages of the trypanosome, impression smears of organs of the infected bats and inoculation of laboratory mice for the presence of crithidial stages of the trypanosome in order to identify them as T. cruzi or "T. cruzi-like."

4. The organisms were identified as Trypanosoma cruzi-like because the morphology was similar to T. cruzi (mean of total length 16.67 microns which is in the range of T. cruzi), because they were incapable of infecting laboratory mice, and because they had no intermediate stages in impressions of organs from the hosts.

5. It was shown that the morphology is affected by the temperature, especially the position of the nucleus and its shape. In conjunction with other types of bats found infected with T. cruzi, this observation supports the hypothesis of Trejos (1963).

6. The ectoparasites present in Tadarida brasiliensis were the same families found in Myotis velifer, although they were of different species, and were present in different proportions. T. brasiliensis was found to be more infested with ectoparasites than Myotis.

7. The fleas (Myodopsylla gentilis) of Myotis were found infected with Leptomonas sp., which apparently is only found in insects, and which constitutes a new record for the host flea.

8. The examination of two triatomids (Triatoma sanguisuga) found near the cave were negative for the presence of flagellates.

9. It is possible that the difference in infection in both species of bats was due to two factors: immunological resistance in Tadarida against this particular strain, and greater susceptibility of Myotis to this trypanosome strain than Tadarida.

10. It was stated that all the ectoparasites found in both species of bats could be potential vectors of the Trypanosoma, although this was not definitely determined, and the bats could acquire an infection by eating infected triatomids or by the bites of bugs found in the entrances of the caves.

CHAPTER V

SUGGESTIONS FOR FURTHER STUDIES

Due to the fact that trypanosome studies in bats are extremely complicated, we suggest that for further studies this work could be divided into three major groups: first from the entomological, second from the biological, and third from the microbiological points of view.

In entomological studies we suggest a survey of triatomids found in Oklahoma especially in those counties near Texas, and the counties in which Myotis velifer are found. Uninfected triatomids should be reared in the laboratory, xenodiagnosis made on infected bats, and the life cycle and stages developed in the insects should be studied. Also an exhaustive study of ectoparasites making appropriate cultures insect flagellates is recommended (Wallace, 1963).

In biological studies we suggest culture in a diphasic media and tissue culture of the flagellates found in bats and fleas, and the comparison of them with those found in ectoparasites. Also we suggest histological sections and impression smears of organs of the bats infected.

In microbiological studies we suggest animal inoculation (mice CFW or C3H) with trypanosomes found in bats and with culture forms found in insects. Also clean bats should be inoculated with infected blood from other bats. The mouse protection test (D'Allessandro, 1963) must be carried out along with studies of the determination of pathogenicity

and serological studies of all the strains of trypanosomes found. The different types of trypanosomes should be compared, and a complement fixation test (Davis, 1946), precipitation test, haemoaglutination, fluorescent antibody techniques (Sadun, 1963), etc. could be included in this work. Immunological studies could be done using Tadarida brasiliensis infected with the same strain found in Myotis. It is recommended that if another student attempts a study of this kind that it should be extended over one or two years, with collections of bats in all counties of Oklahoma in which Myotis velifer and Tadarida brasiliensis are present.

BIBLIOGRAPHY

Anonymous. 1956. Found: Two cases of Chagas' Disease. Texas Health Bull. 9:11-13.

Bagby, J. R., Jr. 1962. Comparative parasitemias and tissue infectivity of several strains of Trypanosoma cruzi Chagas' 1909 in young CFW mice. Doctoral Thesis, Emory University.

Bice, D. E. 1966. The incidence of Trypanosoma cruzi in Triatoma of Tucson, Arizona. Rev. Biol. Trop. 14(1):3-12.

Brooke, M. M., L. Norman, D. Allain, and G. W. Gorman. 1957. Isolation of Trypanosoma cruzi-like organisms from wild animals collected in Georgia. J. Parasitol. 43:15.

Clark, T. B. 1959. Comparative morphology of four genera of Trypanosomatidae. J. Protozool. 6(3):227-232.

Cooley, N. R. 1967. Personal communication.

D'Alessandro, A. B. 1963. The life cycle of Trypanosoma rangeli in Triatomid bugs as it occurs in nature. Bull. Tulane Univ. Med. Fac. 23(1):21-30.

Davis, B. S. 1952. Studies on the trypanosomes of some California mammals. Univ. Calif. Publs. Zool. 57:145-249.

Davis, D. J. and T. de S. Sullivan. 1946. Complement fixation tests for American Trypanosomiasis in Texas. Publ. Health Rep. 61:1083-1084.

Deane, M. P. and E. Kirchner. 1962. Metodo simples de enriquecimento para evidenciar trypanosomas no sangue. Revta. Inst. Med. Trop. 4(6):407-408.

Deane, M. D. and E. Kirchner. 1963. Life cycle of Trypanosoma conorhini. Influence of temperature and other factors in growth and morphogenesis. J. Protozool. 10(4):391-399.

Dias, E. 1940. Sobre um Schizotrypanum dos morcegos Lonchoglossa ecaudata e Carollia perspicillata do Brasil. Mem. Ins. Oswaldo Cruz. 35(2):399-409.

_____, and F. Pifano. 1941. Estudo experimental de um Schizotrypanum do morcego Hemiderma perspicillatum da Venezuela. Mem. Ins. Oswaldo Cruz. 36(1):79-99.

Glass, B. P. and Claud M. Ward. 1959. Bats of the genus Myotis from Oklahoma. J. Mamm. 40(2):194-201.

Herman, C. M. and J. I. Bruce, Jr. 1962. Occurrence of Trypanosoma cruzi in Maryland. Proc. Helm. Soc. Wash. 29:55-58.

Hoare, C. A. 1964. Morphological and taxonomic studies on mammalian trypanosomes. X. Revision of the systematics. J. Protozool. 11(2):200-207.

Kagan, I. G. and L. Norman. 1962. Immunologic studies on Trypanosoma cruzi. IV. Serial transfer of organisms from immune to nonimmune mice. J. Parasitol. 48:584-588.

_____. and D. Allain. 1966. Studies on Trypanosoma cruzi isolated in the United States: a review. Rev. Biol. Trop. 14(1):55-73.

Lesser, E., Burns, K. F., and Farinacci, C. 1957. A blood parasite from the bat Tadarida brasiliensis mexicana. J. Parasitol. 43:380.

Levine, N. D. 1961. Protozoan parasites of domestic animals and of man. Burgess Publishing Co., Minneapolis. 212pp.

Marinkelle, C. J. 1966. Observations on human, monkey, and bat trypanosomes and their vectors in Colombia. Trans. R. Soc. Trop Med. Hyg. 60(1):109-116.

_____. 1967. Personal Communication.

Mitchell, O. G. 1956. Trypanosoma vespertilionis from some southern California bats. J. Mamm. 37(3):443-444.

McKeever, S., F. W. Gorman, and L. Norman. 1958. The occurrence of a Trypanosoma cruzi-like organism in some mammals from southwestern Georgia and northwestern Florida. J. Parasitol. 44:583-587.

Norman, L., M. M. Brooke, D. S. Allain, and F. W. Gorman. 1959. Morphology and virulence of Trypanosoma cruzi-like hemoflagellates isolated from wild mammals in Georgia and Florida. J. Parasitol. 45:457-463.

Olsen, P. F. 1965. The epizoology of Chagas' Disease in the southeastern United States with particular emphasis on Trypanosoma cruzi, Triatoma sanguisuga, Didelphis marsupialis complex in Alabama. (Thesis, Auburn University). Dissertation Abstracts. 26:1235-36.

_____, J. P. Shoemaker, H. F. Turner, and K. L. Hays. 1964. Incidence of Trypanosoma cruzi (Chagas) in wild vectors and reservoirs in East Central Alabama. J. Parasitol. 50:599-603.

Packchanian, A. 1943. On the viability of various species of Trypanosoma and Leishmania cultures. J. Parasitol. 29:275-277.

Sadun, E. H., R. E. Duxbury, J. S. Williams and R. I. Anderson. 1963. Fluorescent, antibody test for the xenodiagnosis of African and American Trypanosomiasis in man. J. Parasitol. 49:385-389.

Sullivan, T. D., T. McGregor, R. B. Eads, and D. J. Davis. 1949. Incidence of Trypanosoma cruzi Chagas' in Triatoma (Hemiptera: Reduviidae) in Texas. Am. J. Trop. Med. 29:453-458.

Taliaferro, W. H. 1923. A study of size and variability, throughout the course of "pure line" infections, with Trypanosoma lewisi. J. Exp. Zool. 37:127-168.

_____. 1924. A reaction product in infections with Trypanosoma lewisi which inhibits the reproduction of the trypanosomes. J. Exp. Med. 39:71-190.

Tobie, E. J. 1964. Cultivation of mammalian trypanosomes. J. Protozool. 11(3):418-423.

Trager, L. W. Jr. 1958. A survey of certain wild rodents for the presence of American Trypanosomiasis in Oklahoma. M. S. Thesis, Oklahoma State University.

Trejos, A., G. A. Godoy, G. Greenblatt, and R. Cedillos. 1963. Effects of temperature on morphologic variation of Schizotrypanum cruzi in tissue culture. Exp. Parasitol. 13:211-218.

Twente, J. W. Jr. 1955. Some aspects of habitat selection and other behavior of cavern-dwelling bats. Ecology. 36(4):706-732.

Van Den Berghe, L. and L. Chardome. 1963. An African bat trypanosome in Stricticimex brevispinosus. J. Protozool. 10:135-138.

Wallace, F. G. 1963. Criteria for the differentiation of genera among the trypanosomid parasites of insects. Progress in Parasitology. First International Congress on Protozoology Prague. pp. 70-74.

_____. 1966. The trypanosomid parasites of insects and arachnids. Exp. Parasitol. 18(1):124-193.

_____. 1967. Personal Communication.

_____. and C. A. Hoare. 1966. Developmental Stages of trypanosomatid flagellates: a new terminology. Nature. 212:1385.

Walton, B. C. 1956. Identification of a Trypanosoma from Procyon lotor lotor from Maryland vs Trypanosoma cruzi (Chagas'). Dissertation Absts. 16:2568.

Wenyon, C. M. 1926. Protozoology. Vol. 1. William Wood and Co., New York. 778pp.

Wood, F. D. and Sherwin F. Wood. 1937. Occurrence of Haematozoa in some California birds and mammals. J. Parasitol. 23:197-201.

Wood, S. F. 1943. A new locality for Trypanosoma vespertilionis (=T. cruzi?) in bats in the United States. J. Parasitol. 29:263.

_____. 1949. Additional observations on Trypanosoma cruzi Chagas' from Arizona in insects, rodents and experimentally infected animals. Am. J. Trop. Med. 29:43-55.

_____. 1952. Mammal blood parasite records from southwestern United States and Mexico. J. Parasitol. 38:85-86.

Woody, N. C. and J. B. Woody. 1955. American Trypanosomiasis (Chagas' Disease). First indigenous case in the United States. J. A. M. A. 159:676-677.

World Health Organization. 1960. Chagas Disease. Techn. Rep. Ser. Wld. Hlth. Org. No. 202. 21pp.

Yaeger, R. G. 1960. A method of isolating trypanosomes from blood. J. Parasitol. 46:288.

VITA

Augusto Alberto Cadena

Candidate for the Degree of

Master of Science

Thesis: STUDIES ON FLAGELLATES OF THE BATS Tadarida brasiliensis AND Myotis velifer AND ON THEIR ECTOPARASITES IN OKLAHOMA

Major Field: Zoology

Biographical:

Personal Data: Born in Bogota, Colombia, August 6, 1938, the son of Alberto and Conchita Cadena.

Education: Graduated from Colegio Santo Tomas de Aquino, Bogota, Colombia, in 1958; received Bachelor of Science degree from the Andes University, with a major in Biology, in August, 1964; completed requirements for the Master of Science degree in July, 1967.

Professional Experience: Graduate teaching assistant, Andes University, Bogota, Colombia, 1964-65; Research assistant, Andes University, Bogota, Colombia, 1963-65; instructor in Biology, Javeriana University, Bogota, Colombia, 1964-1965; Biology teacher, Cundinamarca College, Bogota, Colombia, 1964.

Member: American Society of Mammalogists.