THE TABANIDAE ASSOCIATED WITH WHITE-TAILED DEER IN DIFFERENT HABITATS OF OKLAHOMA AND THE PREVALENCE OF TRYPANOSOMES RESEMBLING *TRYPANOSOMA THEILERI* LAVERAN IN SELECTED SPECIES

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CHAPTER I

INTRODUCTION

The horse flies and deer flies (Diptera: Tabanidae) are a large group of hematophagous flies. Females of most species are voracious and persistent blood feeders that will, when disturbed, often return to the same or another animal to finish feeding, and many species may ingest more than one blood meal. Therefore, the tabanids have the potential to mechanically and biologically transmit diseases. Krinsky (1976) discussed the role of the Tabanidae in the transmission of numerous disease agents including those responsible for hog cholera, anaplasmosis, anthrax, tularemia and brucellosis, all of which are important diseases of North American livestock.

Tabanid feeding activity with its associated blood loss and annoyance may also cause economic loss in livestock. Estimates of blood meal size and attack rates on cattle indicate that blood loss may be substantial in some areas (Tashiro and Schwardt, 1949; and Hollander and Wright, 1980b). Bruce and Decker (1951) reported reduced butterfat production in dairy cattle and reduced weight gains in beef cattle due to tabanid attack. Bruce and Decker (1951) and Roberts and Pund (1974) reported reduced weight gains in beef cattle as a result of the feeding activity of tabanids and other biting flies.

Numerous tabanid surveys have been done in various parts of the

United States, and several authors have surveyed Oklahoma Tabanidae (Schnorrenberg, 1932; Howell and Schomberg, 1955; Hollander, 1979; Ehrhardt, 1981; and Wright et al., 1984). However, with the exception of studies by Dale and Axtell (1976) in North Carolina and Thompson (1977b) in Texas, comparative studies of the adult tabanid fauna in different habitats are scarce. The role of wild mammals as a blood source for female Tabanidae is also poorly understood.

Since Nöller (1925) recovered trypanosomes resembling Trypanosoma theileri Laveran from horse flies in Germany, the Tabanidae have been implicated in the transmission of this species and the related T. cervi Kingston and Morton by numerous authors world-wide. Trypanosomes have been recovered from tabanids by several workers in the United States, and Krinsky and Pechuman (1975) surveyed the incidence of trypanosomes in horse flies and deer flies from New York. However, little is known about the biology of T. theileri and T. cervi in tabanids.

This study had four major objectives:

1. To compare the tabanid faunas of two adjacent but distinct habitats.
2. To determine the role of white-tailed deer (Odocoileus virginianus Rafinesque as a blood source for female tabanids.
3. To survey tabanids for the prevalence of Trypanosoma spp. in an area endemic for T. cervi.
4. To determine the biology of T. theileri in adult female tabanids.

Secondarily, the study was designed to provide information for

a current state survey of the Tabanidae, and to demonstrate the potential of FIAX® (Fluorescent Immunoassay, International Diagnostic Technology) in the study of trypanosom-like organisms in arthropod vectors

CHAPTER II

REVIEW OF THE LITERATURE

Tabanid Surveys

There have been several tabanid surveys in Oklahoma. Schnorrenberg (1932) presented a key to 24 Oklahoma tabanid species and included a discussion of some aspects of the biology of a few species. Howell and Schomberg (1955) presented a checklist of 70 Oklahoma species in nine genera. In recent studies, daily activity patterns (Hollander and Wright, 1980a), blood meal size and preferred feeding sites on cattle (Hollander and Wright, 1980b) and seasonal occurrence (Wright et al., 1984) have been described for the Tabanidae of north central Oklahoma. Ehrhardt (1981) collected 32 tabanid species in southeastern Oklahoma and discussed the parity and ovarian development of four species.

A number of studies have also been done in states bordering Oklahoma. Thompson (1973a, 1973b, 1974a, 1974b, 1976, 1977a and 1977b) described the tabanid faunas of various ecosystems in Texas. Tidwell (1973) completed a systematic study of the Tabanidae of Louisiana which included biological and distributional notes for most species in that state. Schwardt and Hall (1930) produced an incomplete key to Arkansas tabanids by amending an earlier key to Louisiana tabanids (Hine, 1907), and Schwardt (1936) discussed the

life history of 22 tabanid species occurring in Arkansas. Andrews and Wingo (1975) presented a key to the tabanids of Missouri and included a brief discussion of the relative abundance, seasonal occurrence and geographic distribution of most species.

Comparative studies of the adult tabanid faunas of different habitats are relatively scarce. Thompson (1977b) compared the faunas of an upland and lowland ecosystem in south central Texas. He collected 26 species in each habitat, 21 of which were common to both habitats. Slightly more flies were collected at the upland site than at the lowland site, but Thompson felt the lowland fauna was slightly more diverse than that of the upland site.

Dale and Axtell (1976) compared the tabanid faunas of two salt marsh habitats in North Carolina, and found that both abundance and diversity was greater in Spartina marshes than in Juncus marshes. Mullens and Gerhardt (1980) compared the tabanid faunas of three major geographic regions of Tennessee (the Cumberland Plateau, Tennessee Valley and Blue Ridge Mountains). They found several species to be unique to higher altitudes, but that those species common to lower elevations also occurred in higher altitudes.

Trypanosoma spp.

Theiler isolated large numbers of an unidentified Trypanosoma sp. from cattle in South Africa and sent blood smears to A. Laveran who named it Trypanosoma theileri in 1902 (Wells, 1972). The new species immediately aroused considerable interest because Theiler (1903) incriminated it as the causative agent of gall sickness of cattle in Africa, but Theiler (1910) later determined that the

causative agent was actually Anaplasma marginale Theiler. However, as a result of further studies of the trypanosome, it was apparent that T. theileri was a ubiquitous parasite of domestic cattle (Bos taurus L.), and in his review of the literature, Hoare (1972) cited reports of its recovery from the zebu (B. taurus), European bison (Bison bonasus L.), water buffalo (Bubalis bubalis L.), chevrotain (Hyemoschus aquaticus [Ogilby]) and several African antelope species. Crawley (1910) made the first isolation of T. theileri from North American cattle. However, he called it a new species, T. americanum. Trypanosoma theileri apparently occurs in cattle throughout North America and has been reported from Maryland (Crawley, 1910; and Dikmans et al., 1957), Kansas (Ewing and Carnahan, 1967), Illinois (Levine et al., 1956), Wyoming (Mathews, 1975), Florida (Ristic and Trager, 1958), New York (Schlafer, 1979) and Ontario, Canada (Woo et al., 1970). Trypanosoma theileri infections have also been diagnosed in Oklahoma cattle at the Oklahoma State University Veterinary Medicine Hospital.

Trypanosoma theileri has apparently evolved with its bovine host and is normally non-pathogenic. However, several workers have implicated it with various pathological symptoms. Cross et al. (1968) reported lymphocytosis, but no other apparent pathologic change, in cattle in the acute initial phase of infection. Ristic and Trager (1958) recovered T. theileri from dairy cattle with depressed milk production. McLoughlin and Chute (1975) found it in the vaginal washings of a Herford cow experimentally infected with Tritrichomonas foetus (Riedmuller). Levine et al. (1956) recovered T. theileri from a Hereford heifer that had recently aborted, and Dikmans et al.

(1957) recovered it from the stomach of an aborted fetus. Kingston et al. (1982) reported that pregnant cows experimentally infected with T. theileri exhibited a disproportionately high abortion rate.

The trypanosomes of North American cervids have been reported by all investigators as being morphologically similar to T. theileri. Kistner and Hanson (1969) reported trypanosomes in white-tailed deer from Alabama, Florida, Georgia, North Carolina, South Carolina and Virginia. Clark (1972) reported trypanosomes in mule deer Odocoileus hemionus (Rafinesque) from New Mexico and Colorado. Kingston and Morton (1973) recovered trypanosomes from elk (Cervus elaphus nelsoni Bailey) and Kingston et al. (1975) from mule deer in Wyoming. Kingston and Morton (1975) described the trypanosome from elk as a new species, Trypanosoma cervi, based on the morphological characters of the flagellum and their inability to get cross-infections with cattle. Mathews et al. (1977) demonstrated that the trypanosomes infecting elk and mule deer in Wyoming were the same species. Based on morphological measurements, Kingston and Crum (1977) concluded that the trypanosomes of white-tailed deer in the southeastern United States were conspecific with T. cervi. It is likely that the trypanosome of all North American cervids is T. cervi, and other reports of this parasite include isolations from white-tailed deer from Michigan (Stuht, 1973), New York (Krinsky, 1975) and 10 southeastern states (Davidson et al., 1983). Dr. A. A. Kocan (Oklahoma State University Department of Veterinary Parasitology, Microbiology and Public Health) in personal communication has indicated T. cervi is a common parasite of white-tailed deer throughout Oklahoma. Other reports of T. cervi isolations from elk include Davies and Clark (1974) in New Mexico and

Stuht (1975) in Michigan. There have been no reports of pathological symptoms associated with T. cervi infections.

The primary vectors of both T. theileri and T. cervi are apparently various species of tabanids. In Germany, Nöller (1916) reported flagellates from the hindguts of female tabanids which were indistinguishable from T. theileri in culture, and Nöller (1925) produced a T. theileri infection in a calf by inoculating it with trypanosomes recovered from the hindguts of Haematopota pluvialis L. Kraneveld (1931) produced a T. theileri infection in cattle by placing dissected gut material from Tabanus striatus Fabricius on the oral mucous membrane. Hoare (1972) and Wells (1972) cite numerous reports of T. theileri isolations from various tabanid species world-wide. Trypanosoma theileri was first recovered from tabanids in North America by Packchanian (1957), who isolated it from Tabanus atratus Fabricius. Clark (1972) reported trypanosomes in the hindguts of tabanids from an area in which there were infected mule deer, and he reported on the possibility of experimental transmission of trypanosomes to elk inoculated with material from the heads of tabanids. Davies and Clark (1974) found the trypanosomes they recovered from horse flies in Wyoming to be almost identical to those found in elk in the same area and presumed they were the same species. Krinsky and Pechuman (1975) recovered trypanosomes resembling T. theileri from 24 tabanid species in New York, and they speculated the flies may have acquired them from either infected deer near the collection site or from dairy cattle.

While various tabanid species are probably the normal vectors of T. theileri and T. cervi, Burgdorfer et al. (1973) recovered T.

theileri from two species of ixodid ticks in Ethiopia, and Krinsky and Burgdorfer (1976) recovered trypanosomes resembling T. theileri from lone star ticks (Amblyomma americanum) in the Cookson Hills Refuge, Cherokee County, Oklahoma. There is evidence that T. theileri can also be transmitted to the bovine fetus transplacentally. Lundholm et al. (1959) reported T. theileri as a contaminant of fetal bovine kidney cells, and Dikmans et al. (1957) recovered it from the stomach of an aborted bovine fetus. Kingston et al. (1982) found trypanosomes in a fetus taken by Caesarean section from a cow which had been experimentally infected with T. theileri during pregnancy.

CHAPTER III

THE TABANIDAE (DIPTERA) OCCURRING IN TWO ADJACENT HABITATS IN NORTH CENTRAL OKLAHOMA AND THEIR RELATIONSHIP WITH WHITE-TAILED DEER

Introduction

Although there have been several tabanid faunal studies in Oklahoma and surrounding states, there has been relatively little research of the relationship between Tabanidae and different habitats within an ecosystem. Schnorrenberg (1932) surveyed the Tabanidae of Oklahoma and Howell and Schomberg (1955) presented a checklist of 70 species in nine genera occurring in the state. Wright et al. (1984) surveyed the tabanids of north central Oklahoma, as did Ehrhardt (1981) in southeastern Oklahoma. Thompson (1973a, 1973b, 1974a, 1974b, 1976, 1977a and 1977b) described the tabanid fauna in various ecosystems in Texas. Tidwell (1973) completed a key to the Tabanidae of Louisiana describing habitats in which each species was found. Andrews and Wingo (1975) did a similar study in Missouri.

This study was undertaken to compare the tabanid species of two adjacent habitat types within the postoak-blackjack ecosystem of north central Oklahoma as described by Duck and Fletcher (1943).

Materials and Methods

Tabanidae were collected in modified malaise traps described by

Hollander and Wright (1980a) twice weekly from April 17 through July 3, and once weekly from July 12 through September 26, 1979. Traps were baited with approximately 2.25 kg of dry ice for each 24 hour collection period. The trap collection chamber was a 1350 ml clear plastic jar fitted with a funnel with an opening measuring approximately 2.5 cm in diameter. The collection chamber contained a dichlorvos resin piece measuring 2.5 cm by 5 cm to quickly kill specimens. Tabanids were identified with the aid of several taxonomic keys, especially Tidwell (1973) and Stone (1938). All species identifications were verified by Dr. L. L. Pechuman of Cornell University, Ithaca, New York.

Periodic observations of tabanids feeding on white-tailed deer (Odocoileus virginianus Rafinesque) were made twice weekly from April 24 through June 5, and once weekly from June 12 through September 9. All tabanids observed feeding during a 15 minute period at the beginning of each hour were recorded according to species. Observations were made on a hand reared yearling male white-tailed deer or on a three year old male deer kept in a large enclosure with 10-20 other deer of both sexes and various ages.

Study sites were chosen to represent two distinct habitats in the vicinity of Lake Carl Blackwell in northern Payne County, Oklahoma. Upland trap sites were located on the north and south sides of Lake Carl Blackwell. Both sites were characterized by upland savannah of native grasses with scattered blackjack and postoak overstory. The trap site on the south side of the lake had been used as cattle pasture the previous year and was within 50 m of a pasture in which there were cattle throughout the study. The trap site on the north side of

the lake had not been grazed for more than five years and the nearest cattle were more than 500 m away. An enclosure measuring approximately 4 hectares that contained a captive herd of 10-20 adult white-tailed deer of both sexes was within 100 m of the trap. Both trap sites were at the edge of tree lines and within 100 m of small, intermittent creeks.

The enclosure in which feeding tabanids were observed in the upland habitat was approximately 100 m northeast of the trap on the north side of the lake and 200 m east of the large enclosure containing the captive deer herd. It was separated from both by a band of trees. The enclosure was made of chain-link fence that measured 9 m by 27 m by 3 m high. The yearling deer was maintained in this enclosure throughout the study except when it was transported to the woodlot site for observation.

The other trap sites (woodlot sites) were located at the west end of Lake Carl Blackwell near its confluence with Stillwater Creek. The area was an abandoned woodlot of black locust (Robinia pseudo-acacia L.) and was characterized by a mixture of various hardwood trees, scrub thickets (wild plum and sumac) and native grasses. The two trap sites were in openings at the edge of tree lines, and were within 300 m of one another. The west site was within 50 m of a small, intermittent creek, and within 100 m of a small cove of the lake that filled with water in late June. The east side was within 300 m of the lake.

The enclosure at the woodlot site was made of five portable cattle panels that measured 9 m each. It was located approximately 100 m south of one trap and 200 m west of the other. It was located

in scattered black locusts next to the small cove described above. There were no cattle in the area during the study period, but the area had been used as pasture the previous summer. The nearest cattle were in a pasture approximately 100 m north of one trap and 200 m north of the other. The tracks of wild deer were regularly observed on the access road leading to the study area. On days when feeding tabanids were to be observed at the woodlot site, the yearling deer was immobilized with 100 mg of Rompum® and transported to the enclosure in a wooden holding box.

Results

Trapping Results

A total of 10,151 tabanids representing 19 species in seven genera were collected in four malaise traps baited with dry ice in 1979 from April 17 through September 26 (Table I). There were 10 species in the genus Tabanus, four species in the genus Chrysops and one species each in the genera Anacimas, Chlorotabanus, Esenbeckia, Hybomitra and Silvius. A total of 8219 tabanids were collected in the two upland traps. The trap on the south side of the lake accounted for 5576 tabanids, and the trap on the north side for 2643 tabanids. The west woodlot trap accounted for 840 tabanids, and the east woodlot trap for 1092 tabanids. The five most abundantly collected species (Hybomitra lasiophthalma [Macquart], Tabanus abactor Philip, T. mularis Stone, T. subsimilis Bellardi and T. sulcifrons Macquart) made up approximately 93% of all tabanids collected.

Tabanus abactor was the species collected most often (5180 flies)

TABLE I

TABANIDS COLLECTED IN MALAISE TRAPS IN TWO DIFFERENT HABITATS IN PAYNE COUNTY, OKLAHOMA FROM APRIL 17 - SEPTEMBER 25, 1979

Species	Upland Traps	Woodlot Traps	Seasons
Chrysops callidus Osten Sacken	48	35	May 9 - July 20
C. flavidus Wiedemann	41	19	June 13 - Sept. 25
C. pikei Whitney - *C. sequax* Williston	25	16	June 4 - Aug. 23
Esenbeckia incisuralis (Say)	2	0	June 13 - Junly 3
Silvius quadrivittatus (Say)	4	4	June 12 - Aug. 16
Anacimas dodgei (Whitney)	0	2	April 26
Chlorotabanus crepuscularis (Bequaert)	1	0	July 26
Hybomitra lasiophthalma (Macquart)	234	179	April 17 - May 25
Tabanus abactor Philip	4543	637	May 31 - Sept. 25
T. atratus Fabricius	16	8	May 25 - Sept. 18
T. equalis Hine	168	134	June 4 - July 12
T. mularis Stone	1594	246	May 25 - Sept. 18
T. nigripes Wiedemann	1	2	July 3 - July 20
T. subsimilis Bellardi	457	335	May 9 - Sept. 25
T. sulcifrons Macquart	1054	246	July 3 - Sept. 25
T. stygius Say	1	0	June 13
T. trimaculatus Palisot de Beauvois	26	59	May 15 - July 20
T. venustus Osten Sacken	4	10	June 12 - Aug. 1
Total	8219	1932	April 17 - Sept. 25

and accounted for 50.5% of the total tabanid collection. It was first collected May 31 and was active when trapping ceased September 25. It was most abundant in late June through the end of July and its numbers peaked July 12 when 727 specimens were collected (Figure 1). More than seven times as many T. abactor were collected in the upland traps as in the woodlot traps (4543 and 637, respectively).

Tabanus mularis Stone was the second most abundantly collected species with a total catch of 1840 specimens, accounting for 17.9% of the total tabanid collection. It was most abundant in June and July, and its numbers were greatest June 12 when 644 flies were collected (Figure 2). Approximately 6.5 times as many T. mularis were collected in the upland traps (1594) as in the woodlot traps (246).

A total of 1408 T. sulcifrons were trapped. It was the third most abundantly collected species and comprised 13.7% of all tabanids collected. It first appeared July 3 and was last collected September 25. It was most numerous August 10 when 283 were collected, but it also appeared to have an early population peak in July (Figure 3). Approximately three times as many T. sulcifrons were collected in the upland traps as in the woodlot traps (1054 and 354, respectively).

The fourth most abundant species collected was T. subsimilis with a total catch of 792 flies. It was first collected May 9 and was still active September 25 when trapping ceased. This species was most abundant in June, July and early August (Figure 4). Approximately 1.4 times as many T. subsimilis were collected in upland traps (457) as in woodlot traps (335).

Hybomitra lasiophthalma was the fifth most collected species.

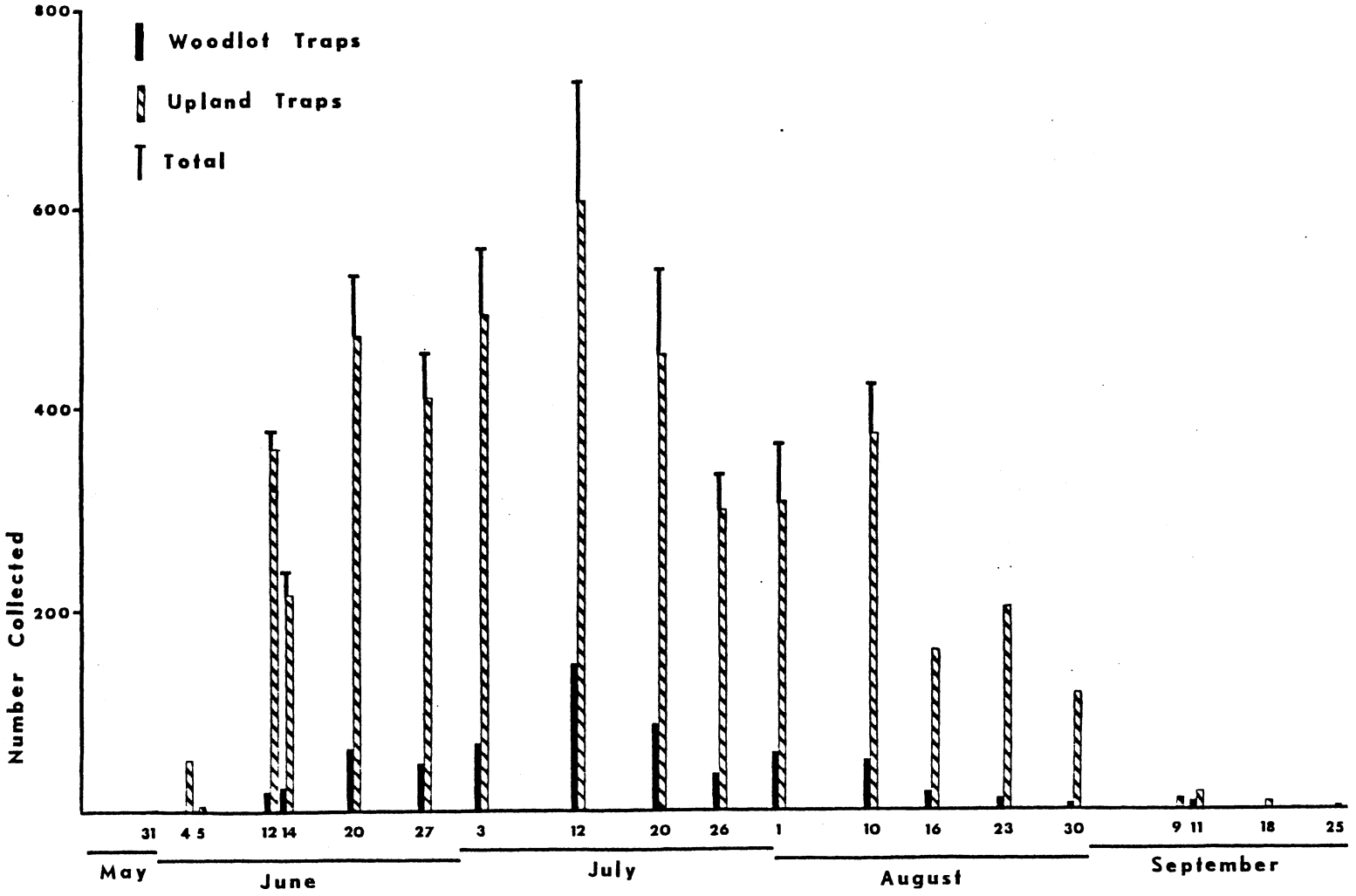

Figure 1. The Seasonal Distributition of Tabanus abactor Collected in Malaise Traps in Two Habitats in Payne County, Oklahoma During 1979.

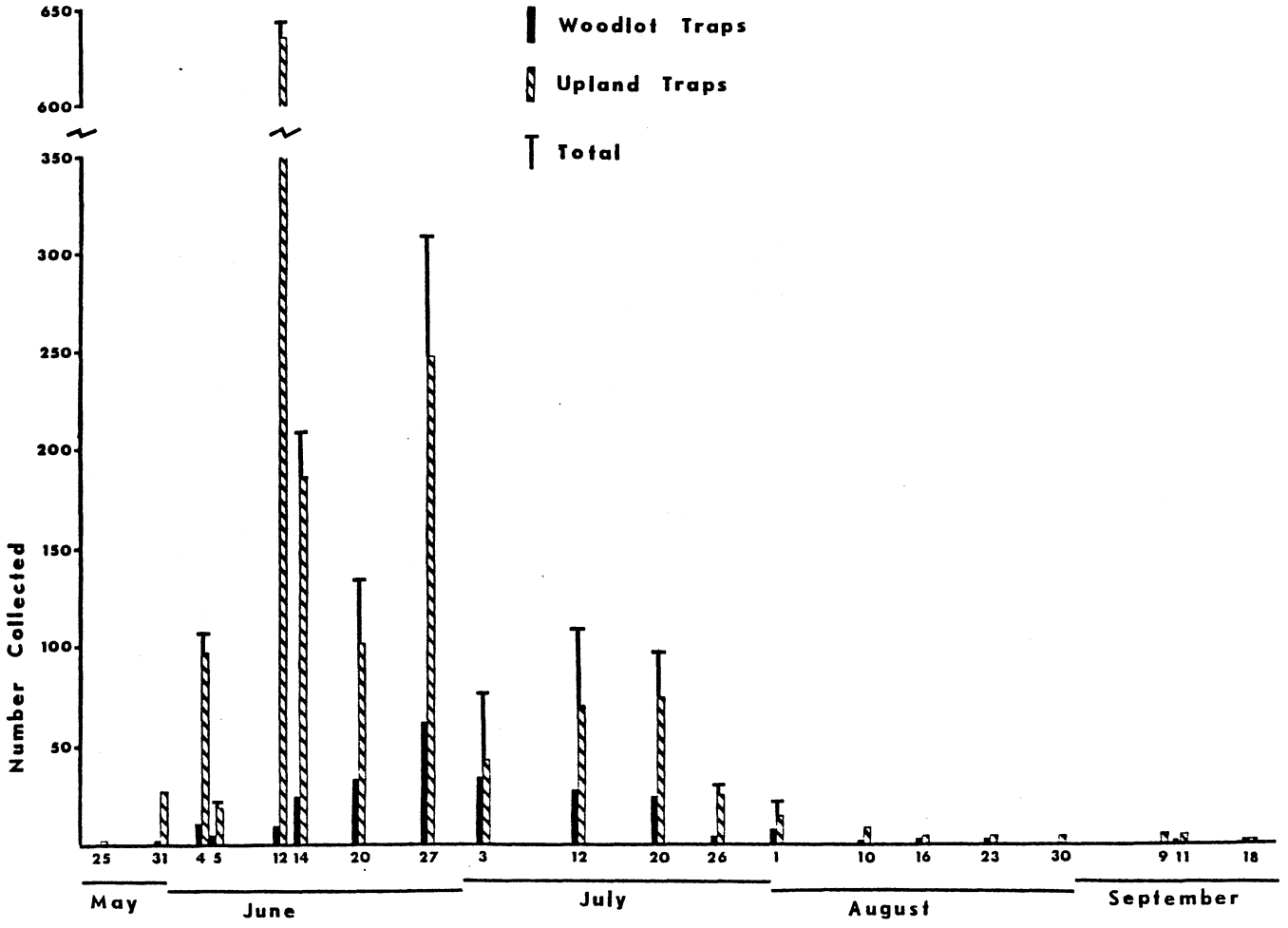

Figure 2. The Seasonal Distribution of Tabanus mularis Collected in Malaise Traps in Two habitats in Payne County, Oklahoma During 1979.

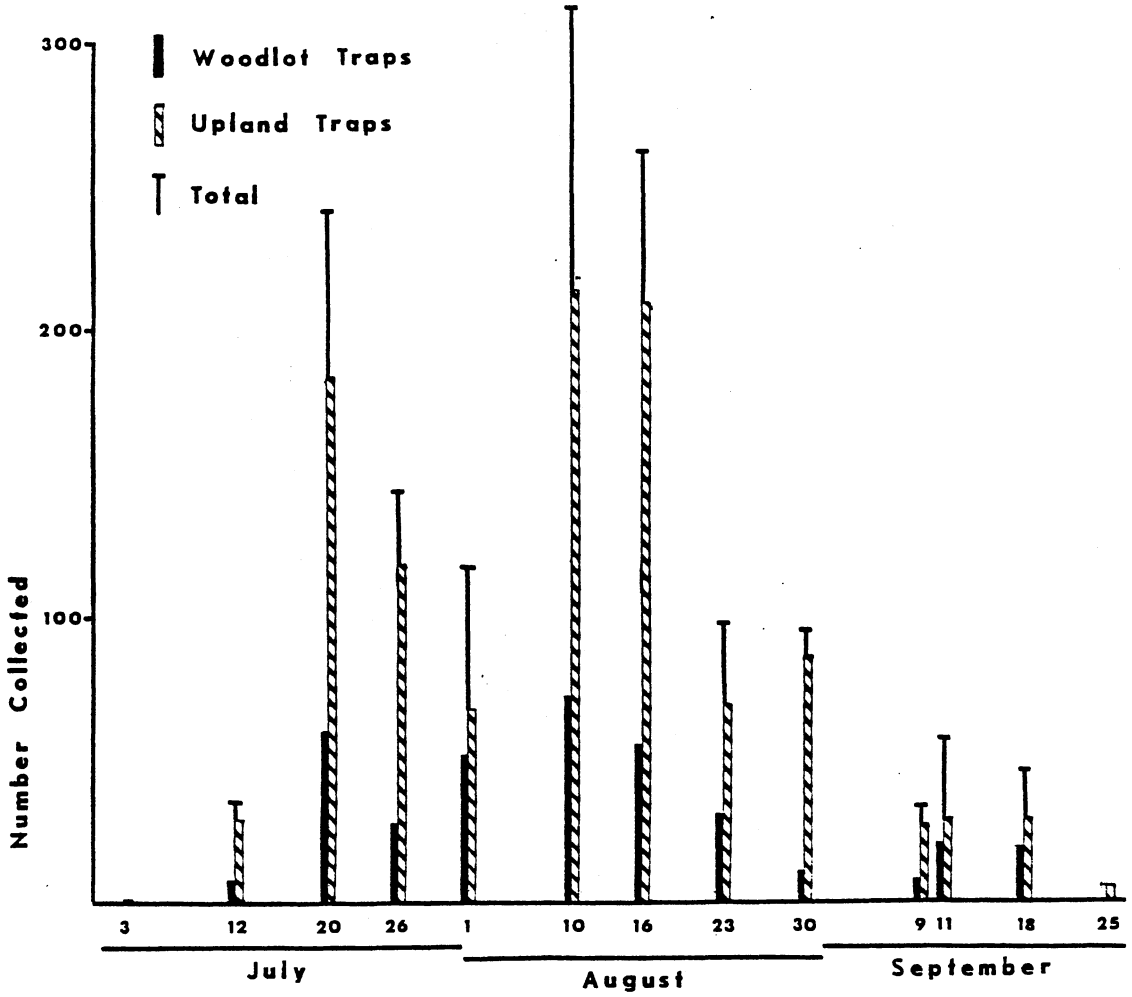

Figure 3. The Seasonal Distribution of Tabanus sulcifrons Collected in Malaise Traps in Two Habitats in Payne County, Oklahoma During 1979.

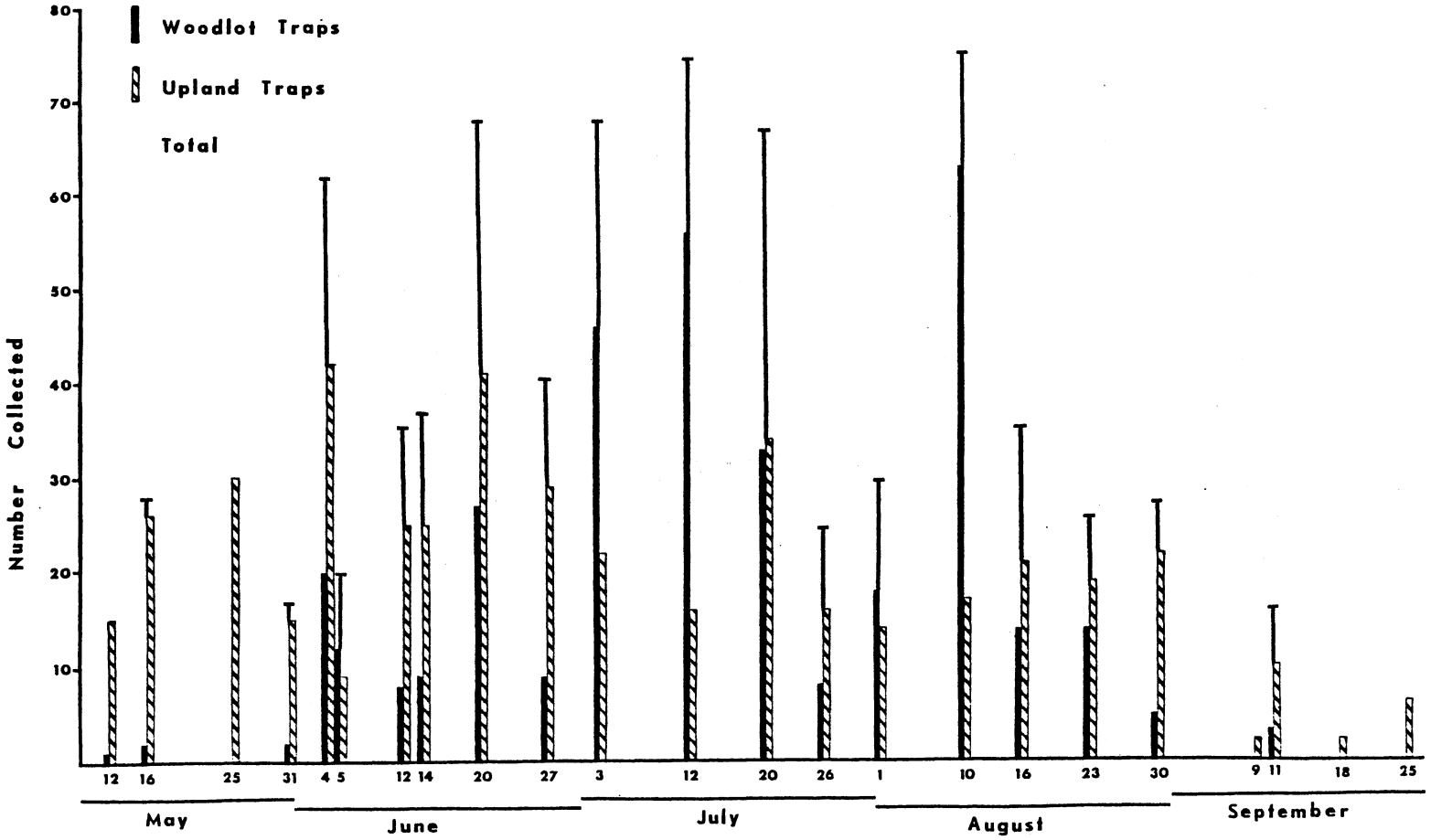

Figure 4. The Seasonal Distribution of Tabanus subsimilis Collected in Malaise Traps in Two Malaise Traps in Two Habitats in Payne County, Oklahoma During 1979.

It was first collected April 17 and last collected May 25. Excessive rain prevented collections during the first week of May. The greatest number of specimens were trapped April 26 (106). It was the first species collected in the spring, and, with the exception of Anacimas dodgei (Whitney), adult emergence ended earlier than any other species (Figure 5). Approximately 1.3 times as many H. lasiophthalma were collected in the upland traps as in the woodlot traps (234 and 179, respectively). The relative abundance of H. lasiophthalma at the two habitats changed between April and May. In April about three times as many were collected in the woodlot traps as in the upland traps, and in May about four times as many were collected in upland traps as in woodlot traps.

Tabanus equalis Hine and T. trimaculatus Palisot de Beauvois were the only other Tabanus spp. for which more than 50 specimens were collected (302 and 85, respectively). Tabanus equalis was first collected June 4, last collected July 12, and the greatest number of specimens were collected June 27 (Figure 6). There were 166 T. equalis collected in upland traps and 136 collected in woodlot traps.

Tabanus trimaculatus was first collected May 15, last collected July 20, and was most numerous on June 12 (Figure 7). Of the seven most abundant species, this was the only one for which more specimens were trapped in the woodlot traps than in upland traps. There were 59 collected in upland traps and 26 collected in woodlot traps. Other Tabanus spp. collected were 24 T. atratus Fabricius, three T. nigripes Wiedemann, one T. stygius Say and 24 T. venustus Osten Sacken (Table I).

Four Chrysops spp. were identified (Table I). Eighty-three C.

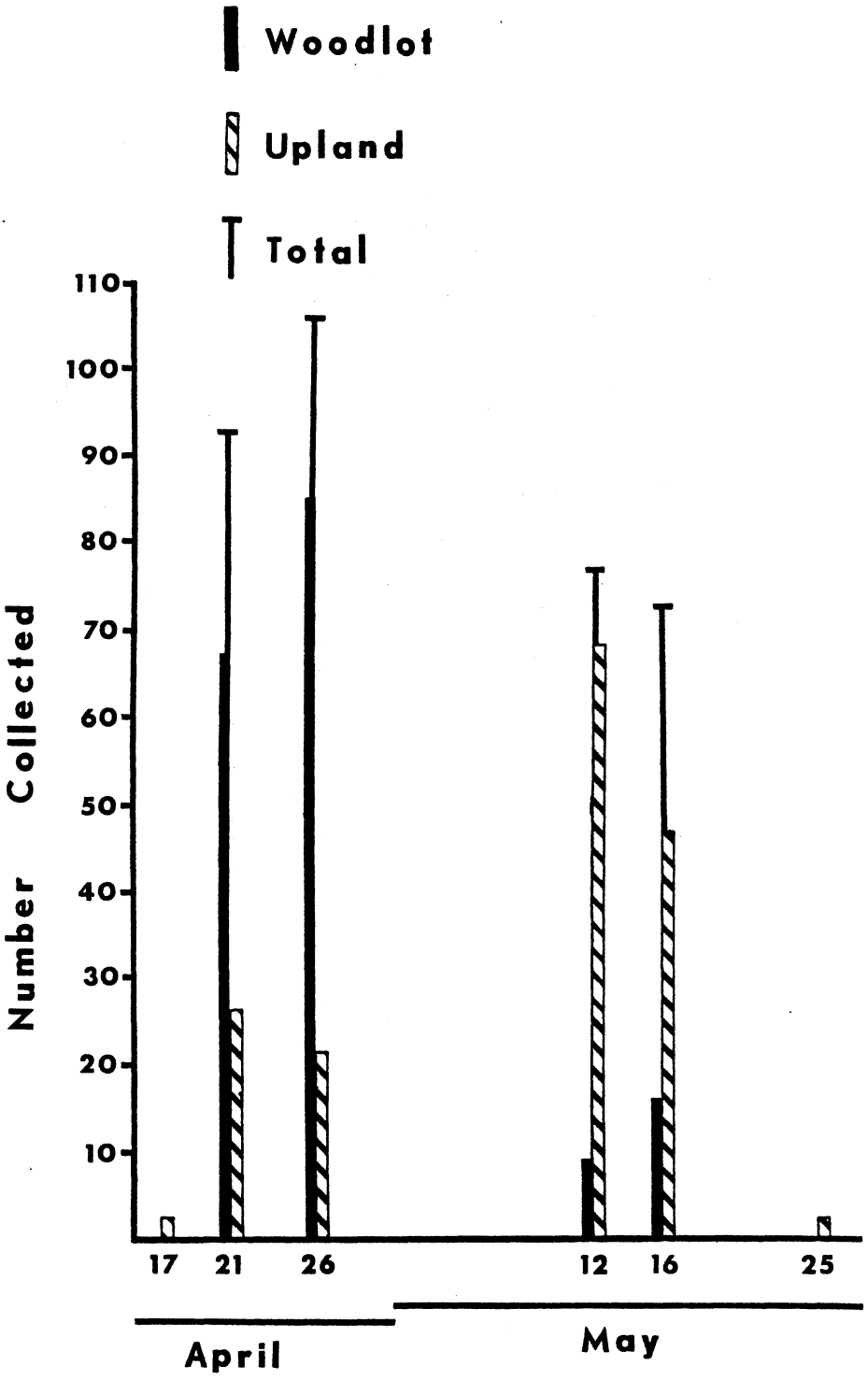

Figure 5. The Seasonal Distribution of Hybomitra lasiophthalma Collected in Malaise Traps in Two Habitats in Payne County Oklahoma During 1979.

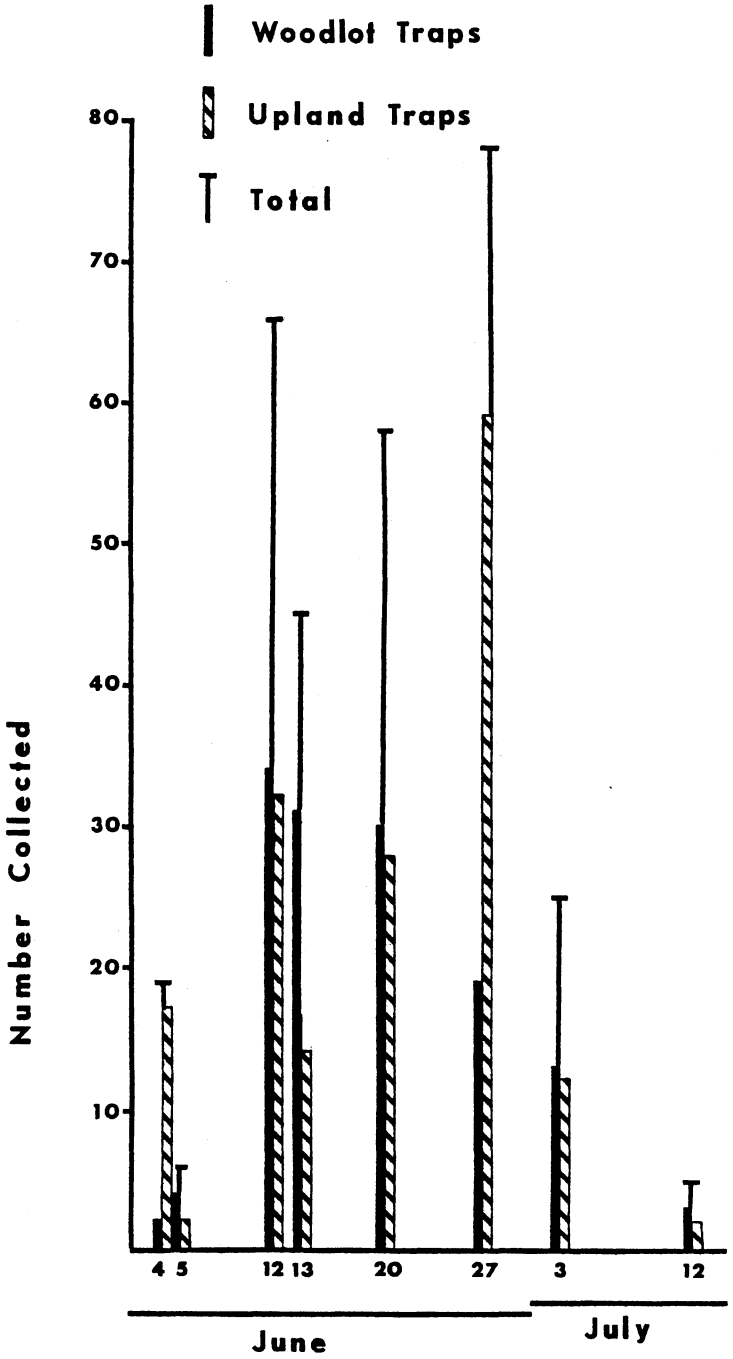

Figure 6. The Seasonal Distribution of Tabanus equalis Collected in Malaise Traps in Two Habitats in Payne County, Oklahoma During 1979.

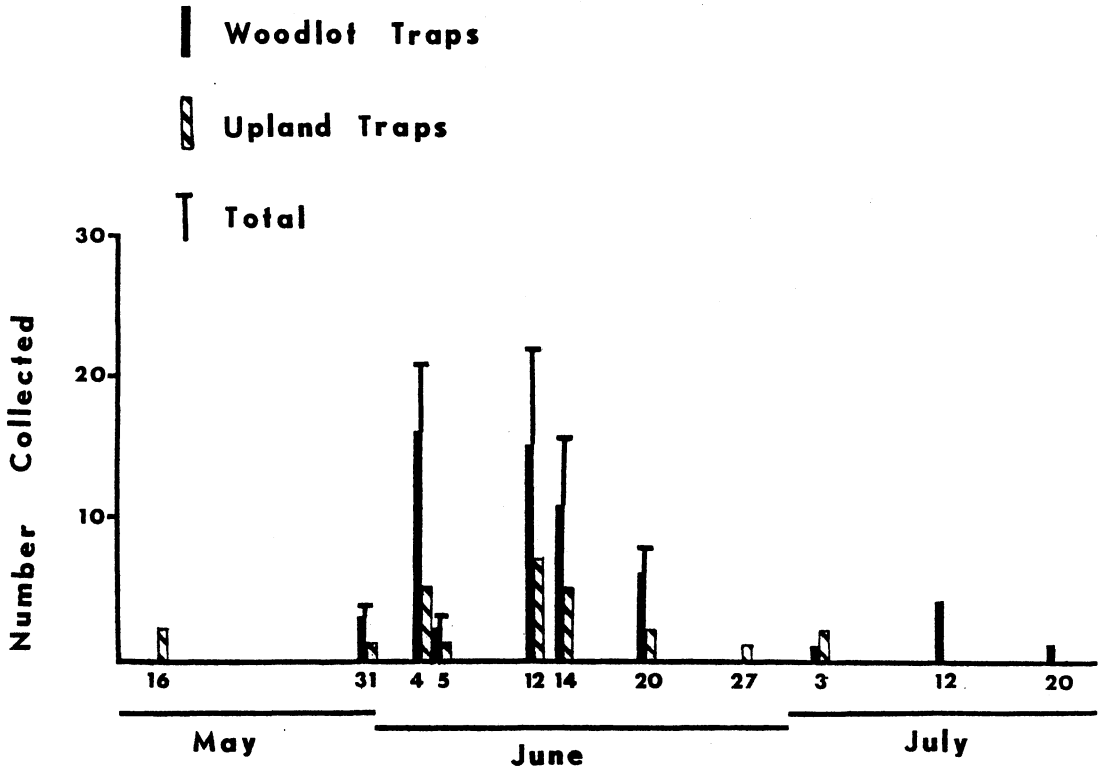

Figure 7. The Seasonal Distribution of Tabanus trimaculatus Collected in Malaise Traps in Two Habitats in Payne County, Oklahoma During 1979.

callidus Osten Sacken were collected from May 9 through July 20. It was most abundant in early June, and it was most numerous June 4 when 21 were collected. There were 48 C. callidus collected in upland traps and 35 in woodlot traps. Sixty C. flavidus Wiedemann were collected from June 13 through September 25 and the highest 24 hour catch was only nine on August 16. More were collected in upland traps than in woodlot traps (41 and 19, respectively). Forty-one specimens originally identified as C. sequax Williston were collected from June 4 through August 30. It was later determined that these were actually two species, C. sequax and C. pikei Whitney. Although the number of each species trapped is unknown, later studies indicate that C. pikei is the more common of the two species. The only other tabanid species collected were two Anacimas dodgei collected on April 26, one Chlorotabanus crepuscularis (Bequaert) collected on July 26, two Esenbeckia incisuralis (Say) collected on June 13 and July 3, and eight Silvius quadrivittatus (Say) collected from June 12 through August 16 (Table I).

Direct Observations of Tabanidae Feeding on White-tailed Deer

A total of 243 tabanids representing nine species in three genera were observed feeding on a white-tailed deer during 265 observation periods of 15 minutes each (0.92 flies/period). In the small upland enclosure 124 feeding tabanids were observed during 147 observation periods (0.84 flies/period); at the woodlot site 47 feeding tabanids were observed during 110 periods (0.42 flies/period); and in the 4 hectare upland enclosure 72 feeding tabanids were observed during eight

periods (9.0 flies/period) (Table II).

The four species most often observed on white-tailed deer were 204 T. mularis, 120 T. abactor, 73 H. lasiophthalma and 24 T. equalis, and they accounted for 95% of all tabanids seen feeding on deer. The visitation rates of these four species in the three observation enclosures are presented in Table III. The other species observed feeding were eight T. subsimilis, seven C. callidus, five T. sulcifrons, one T. atratus and one C. pikei (or C. sequax). Tabanus equalis was the only species that appeared to cause noticeable annoyance. Deer in the 4 hectare enclosure became highly agitated during the crepuscular period when this species was actively feeding.

Discussion

The relative abundance and seasonal activity patterns of the tabanid species collected in this study are similar to that found by Wright et al. (1984), and T. abactor, T. mularis, T. sulcifrons, T. subsimilis, H. lasiophthalma and T. equalis were the five most abundant species collected in both studies. While the relative abundance of the species collected at both sites were similar, consistently more specimens were collected in upland traps than in woodlot traps.

It appears that at least three of the predominant species have evolved in upland habitats. Schomberg and Howell (1955) found the larvae of T. abactor and T. equalis in well drained soils associated with upland trees, and L. M. Cooksey (1983, unpublished data) found T. abactor larvae in similar habitats. Schomberg (1952) recovered a total of 255 T. sucifrons larvae, and all but one were collected in well drained upland soils. The other specimen was collected in

TABLE II

OBSERVED ATTACK RATE OF ALL TABANIDS ON WHITE-TAILED DEER
DURING HOURLY 15 MINUTE OBSERVATION PERIODS
NEAR LAKE CARL BLACKWELL, OKLAHOMA IN 1979

Observation Site	No. of 15 Min. Obs. Periods	No. of Feeding Tabanids	No. of Tabanids per Obs. Period
Woodlot Enclosure	110	47	0.42
Small Upland Enclosure	147	124	0.84
Large Upland Enclosure	8	72	9.00
Total	265	243	0.92

TABLE III

THE OBSERVED ATTACK RATE OF FOUR TABANID SPECIES FEEDING ON WHITE-TAILED DEER IN PAYNE COUNTY, OKLAHOMA DURING HOURLY 15 MINUTE PERIODS

Species	Seasonal[1] Activity	Woodlot Enclosure			Small Upland Enclosure			Large Upland Enclosure			Total		
		No.[2] Obs. Per.	No. Flies Obs.	No. Per.	No. Obs. Per.	No. Flies Obs.	No. Per.	No. Obs. Per.	No. Flies Obs.	No. Per.	No. Obs. Per.	No. Flies Obs.	No. Per.
H. lasiophthalma	4/17 - 5/25	23	7	0.30	50	24	0.48	----	----	----	73	31	0.42
T. abactor	5/31 - 9/25	16	16	1.00	96	75	0.78	8	4	0.50	120	94	0.78
T. equalis[3]	6/4 - 7/12	8	12	1.50	12	0	0.00	4	11	2.75	24	23	0.96
T. mularis	5/25 - 9/18	88	1	0.01	108	13	0.12	8	54	6.75	204	68	0.33

[1] Seasonal activity was determined by the length of time a species was collected in malaise traps.

[2] The number of observation periods for a species was the number of 15 minute periods observed during which a species was seasonally active.

[3] Attack rates for T. equalis were based on observations made between 1900 and 2100 hours, the only hours during which this species was observed on deer.

permanently moist soil associated with a seep spring. Drees et al. (1980) and Pechuman (1972) also reported that the larvae of T. sulcifrons occurred mainly in well drained soil. However, Thompson et al. (1978) and Wilson (1969) reported the collection of a small number of larval T. sulcifrons in semiaquatic habitats. Even though the larvae of T. mularis have never been collected in the field, it seems likely that it too inhabits upland soils. The larvae of T. subsimilis, T. trimaculatus and H. lasiophthalma have all been reported as being collected most often in semiaquatic habitats (Andrews and Wingo, 1975; Pechuman, 1972; Teskey, 1962; Thompson et al., 1978; and Wilson, 1969).

White-tailed deer appear to be of minor importance as a blood meal source for Tabanidae in the study area. The visitation rates of all species of feeding females were insignificant when compared to visitation rates on cattle (Hollander and Wright, 1980a), and only T. equalis appeared to be a source of annoyance to deer. Host preference may be due to behavioral and/or biological characteristics of the host, or to the evolution of the fly/host relationship. White-tailed deer are a woodland species, coming to clearings late in the evening and tend to remain hidden in thickets and forested areas during the day. They also tend to occur alone or in small groups. Cattle mainly feed in grasslands during the day, and they congregate in large groups which may provide a more attractive stimulus to host seeking flies (both visually and chemically), and the deer in the large enclosure, where they were maintained in an artificially large group with little cover, were attacked much more frequently than the single animal observed in the small enclosures. However, the attack

rate on these deer was still much lower than Hollander and Wright (1980b) observed on cattle.

CHAPTER IV

TRYPANOSOMES RESEMBLING *TRYPANOSOMA THEILERI* LAVERAN IN TABANIDAE (DIPTERA) FROM CHEROKEE COUNTY, OKLAHOMA

Introduction

Flagellates resembling *Trypanosoma theileri* Laveran were first reported in Tabanidae by Nöller (1916) in Germany. Nöller (1925) and Kraneveld (1931) recovered *T. theileri*-like organisms from tabanids and apparently produced bovine parasitemias with them. Packchanian (1957) first noted *T. theileri*-like flagellates in tabanids in the United States when he described trypanosomes indistinguishable from *T. theileri* in *Tabanus atratus* Fabricius. *Trypanosoma theileri*-like flagellates have been recovered from tabanids in this country by Clark (1972) who apparently produced a parasitemia in an elk (*Cervus elaphus nelsoni* Bailey) with an injection of the heads and mouthparts of infected flies. Davies and Clark (1974) also reported trypanosomes from tabanids and described them as morphologically similar to those recovered from elk with which they were associated. Kingston and Morton (1975) classified these organisms associated with North American cervids as a new species, *T. cervi*. Krinsky and Pechuman (1975), in an extensive study of the trypanosomes of New York tabanids reported flagellates, most of which resembled *T. theileri*, in 36 tabanid species

representing four genera. They reported infection rates ranging from 14%-57% in three areas of the state over a period of two years.

The present study was undertaken to determine the incidence of T. theileri-like organisms in Oklahoma Tabanidae and to verify the organs in which they are found within the fly. The study also contributed to a survey of the tabanid fauna of Oklahoma.

Materials and Methods

Tabanid Collections

Adult Tabanidae were collected once weekly at the Gruber Wildlife Management Area in Cherokee County, Oklahoma from April 27 through September 20, 1980. Tabanids were also collected on four different days in 1982 between May 3 and August 17. Trapping with a modified malaise trap described by Wright and Hollander (1980a) began no later than 0900 hours and ended at dark or when tabanids became inactive. The trap was baited with CO_2 gas flowing at a rate of 600-1000 ml per minute. The collection chamber was a 3.8 l clear plastic fish bowl vented on two sides to provide ample air circulation. The trap was placed so that it would be in shade during mid-day, and a reflective white hood was fitted to the trap top to provide additional shade for trapped flies.

The study area was within the oak-hickory ecosystem as described by Duck and Fletcher (1943). The trap site was in a bottomland hardwood habitat until September 6. The site was adjacent to a steep hill to the north and small, semipermanent swamp to the west, and was within 500 m of an arm of Greenleaf Reservoir. The swamp was dry

by mid-July. On September 6 the trap site was moved approximately 500 m to the top of the adjacent hill. This site was at the junction of a native grass pasture and a postoak-blackjack oak forest. On September 20 the trap site was moved approximately 4.8 km north of the original site. This site was in a postoak-blackjack oak forest and was 200 m from a small permanent pond.

Tabanids were removed from the trap at 2-3 hour intervals, placed in paper cans and put in an ice chest for transport to the laboratory where they were sorted to species and counted. Flies to be cultured for trypanosomes were then placed in a refrigerator until they were dissected. Tabanids were identified with the use of various taxonomic keys, particularly Brennan (1935), Stone (1938) and Tidwell (1973). Species identifications were verified by Dr. L. L. Pechuman at Cornell University, Ithaca, New York.

Dissection and Culture Procedures

Veal Infusion Medium (VIM) dispensed in 10 ml aliquots into 16 X 125 mm glass culture tubes was used as a culture medium for Trypanosoma species. Penicillin and streptomycin were added to suppress bacterial growth. A tabanid alimentary tract was cultured intact in a culture tube, or it was separated into its respective parts and cultured in separate culture tubes. Groups of 1-10 flies, usually 5 or 10, of a single species were cultured in a single culture tube.

The following procedures were done in sequence when tabanid alimentary tracts were cultured as component parts. First a wing was clipped near its base, hemolymph was drawn into a microcapillary tube,

and the tube was crushed in VIM. The mouthparts were then removed from the fly and placed in a culture tube. If fecal fluid was exuded during these two steps, it was drawn into a microcapillary tube, and the tube crushed in VIM.

Following removal of the mouthparts, the head was cut off and discarded, and an incision extending from the anterior prothorax to the anus was made on the ventral side. The fly was next immersed in physiological saline and the entire alimentary tract, including the salivary glands, was removed intact. Salivary glands, anterior gut and posterior gut were removed in sequence from the dissected alimentary tract and placed in separate VIM tubes. The intact alimentary tracts of some flies were also cultured whole.

After one week of incubation at room temperature, a drop of culture medium was placed on a microscope slide and examined under a microscope at 100X magnification. If no trypanosomes were found, the tube was incubated for another week and re-examined as above. If Trypanosoma spp. were observed in a tube, the tube was recorded as positive for one tabanid regardless of how many flies were in that particular tube.

Results

Tabanid Collections

A total of 3760 tabanids representing 33 species in five genera were collected in a malaise trap baited with CO_2 gas from April 27 through September 20, 1980 (Table IV). There were 21 species collected in the genus Tabanus, seven species in the genus Chrysops, three

TABLE IV

THE SEASONAL DISTRIBUTION OF TABANIDS COLLECTED IN A MALAISE TRAP IN CHEROKEE COUNTY, OKLAHOMA IN 1980

Species	Total	Season
Chrysops callidus Osten Sacken	23	May 19 - August 11
C. flavidus Wiedemann	51	June 2 - July 3
C. pikei Whitney - C. sequax Williston	107	May 13 - Sept. 6
C. moechus Osten Sacken	4	June 2 - June 16
C. separatus Hine	2	May 6 - May 13
C. upsilon Philip	18	July 15 - Sept. 20
Hybomitra difficilis (Wiedemann)	30	April 27 - May 19
H. lasiophthalma (Macquart)[1]	394	April 27 - May 25
H. nigricans (Wiedemanni)	10	April 27 - May 6
Chlorotabanus crepuscularis (Bequaert)	47	June 23 - July 6
Leucotabanus annulatus (Say)	4	June 30
Tabanus abactor Philip	506	June 23 - Sept. 20
T. abdominalis Fabricius	40	July 15 - August 3
T. americanus Forster	4	June 30 - July 15
T. atratus Fabricius	5	July 21 - August 3
T. equalis Hine	303	June 2 - July 27
T. lineola Fabricius - T. subsimilis Bellardi	426	May 6 - Sept. 6
T. melanocerus Wiedemann	8	July 6 - July 27
T. molestus Say	6	June 23 - June 30
T. mularis Stone	358	May 25 - Sept. 20
T. nigripes Wiedemann	5	July 15 - July 21
T. proximus Walker	5	July 21 - August 30
T. pumilus Macquart	6	June 30 - August 30
T. quinquevittatus Wiedemann	2	July 15
T. sparus milleri Whitney	328	June 2 - July 27
T. stygius Say	6	June 30 - July 27
T. sulcifrons Macquart	591	July 6 - Sept. 20
T. trimaculatus Palisot de Beauvois	43	June 2 - July 27
T. venustus Osten Sacken	7	June 23 - July 27
T. wilsoni Pechuman	331	June 2 - Sept. 20
Total	3760	April 27 - Sept. 20

[1] Ten of 80 specimens collected May 6 were males.

species in the genus Hybomitra and one species each in the genera Chlorotabanus and Leucotabanus. The seven most collected species were 591 T. sulcifrons Macquart, 506 T. abactor Philip, 394 H. lasiophthalma (Macquart), 358 T. mularis Stone, 331 T. wilsoni Pechuman, 328 T. sparus milleri Whitney and 303 T. equalis Hine, and they accounted for approximately 76% of all tabanids collected.

Hybomitra difficilis (Wiedemann), H. lasiophthalma and H. nigricans (Wiedemann) were all captured on April 27 and were the first species collected. The latest species to appear were T. proximus Walker and T. atratus which were both collected on July 21. On September 20, the last day the malaise trap was run, T. abactor, T. mularis, T. sulcifrons and T. wilsoni were collected. The two species collected over the longest period of time were T. subsimilis Bellardi (124 days) and T. mularis (119 days).

Species identifications of specimens collected August 3 revealed that what had been called T. subsimilis was actually two species, T. subsimilis and T. lineola Fabricius. A subsequent investigation of preserved flies showed that T. subsimilis had been active since May 6 and T. lineola had been active at least as early as June 2. After the collecting season ended, it was discovered that what had been called Chrysops sequax Williston actually represented both C. sequax and C. pikei Whitney.

The T. lineola collected represent the first verified records of this species from Oklahoma. The T. wilsoni collected represent the third report of this species from Oklahoma. The T. stygius Say represent a southward extension of the reported range for this species (Pechuman et al., 1983) of more than 80 km.

In 1982 a total of 1169 tabanids were collected representing 19 species in three genera (Table V). There were 500 H. lasiophthalma and 310 T. abactor collected, and these two species combined accounted for 69% of the total catch. A total of 98 T. sulcifrons, 69 T. mularis and 60 T. sparus milleri were collected, and these with H. lasiophthalma and T. abactor accounted for 89% of the total catch. There were 23 C. sequax collected, and it was the most abundantly collected Chrysops species.

Trypanosome Cultures

Results from trypanosome culture procedures are presented in Table VI. The alimentary tracts of 1210 specimens representing 17 species in three genera were cultured whole or in sections. Of these, the intact alimentary tracts, including salivary glands of 1033 were dissected and cultured separately. The mouthparts of 492 flies, hemolymph of 617 flies and the fecal fluid obtained from 157 flies during dissection were also cultured.

A total of 53 tabanids (4.4%) were positive for Trypanosoma spp. in VIM cultures. The posterior guts of 38 flies (3.7%), anterior guts of four flies (0.4%) and salivary glands of three flies (0.3%) were positive for trypanosomes. The mouthparts of three flies (0.3%) and fecal fluid of one fly (0.6%) were also positive for trypanosomes. None of the hemolymph cultures were trypanosome positive.

Among the species for which 50 or more flies were cultured, H. lasiophthalma showed the highest incidence of infectivity (7.1%). Trypanosoma spp. were cultured from the posterior gut, anterior gut, salivary glands and mouthparts. The infectivity rate of T. equalis

TABLE V

TABANIDS COLLECTED IN A MALAISE TRAP IN CHEROKEE COUNTY, OKLAHOMA IN 1982

Species	May 3	July 10	Aug. 10	Aug. 17	Total
Chrysops callidus Osten Sacken	1	4	--	1	6
C. flavidus Widemann	--	--	1	1	2
C. pikei Whitney	--	1	--	--	1
C. sequax Williston	--	11	2	10	23
C. upsilon Philip	--	7	3	9	19
Hybomitra difficilis (Wiedemann)	9	--	--	--	9
H. lasiophthalma (Macquart)	500	--	--	--	500
Tabanus abactor Philip	--	49	143	118	310
T. americanus Forster	4	--	--	--	4
T. cymatophorus Osten Sacken	--	--	1	--	1
T. fairchildi Stone	--	--	1	--	1
T. lineola Fabricius	--	22	4	1	27
T. molestus Say	--	4	--	--	4
T. mularis Stone	--	61	2	6	69
T. subsimilis Bellardi	4	6	3	--	13
T. sulcifrons Macquart	--	24	53	21	98
T. sparus milleri Whitney	--	60	--	--	60
T. wilsoni Pechuman	--	17	5	2	24
T. proximus Walker	--	--	--	2	2
Total	518	266	218	171	1173

TABLE VI

INFECTION RATES FOR FLAGELLATES RESEMBLING *TRYPANOSOMA THEILERI* IN TABANIDS COLLECTED IN CHEROKEE COUNTY, OKLAHOMA IN 1980

Species	Entire Alimentary Tract		Component Parts						Total Infectivity	
			Posterior Gut		Anterior Gut		Salivary Glands			
	No. Cult.	No. Pos.	No. Cult.	No. Pos.	No. Cult.	No. Pos.	No. Cult.	No. Pos.	No. Cult.	No. Pos.
Chrysops callidus	1	0	3	0	3	1(33.3%)	3	0	4	1(25.%)
C. flavidus	0	0	14	0	14	0	14	0	14	0
C. pikei & *C. sequax*	15	3(20.0%)	12	0	12	0	12	0	27	3(11.1%)
Hybomitra difficilis	0	0	21	4(19.0%)	21	0	21	0	21	4(19.0%)
H. lasiophthalma	0	0	255	16(6.3%)	255	2(0.8%)	255	1(0.4%)	255	18(7.1%)
Tabanus abactor	7	1(14.3%)	54	3(5.6%)	54	0	54	1(1.9%)	61	4(6.6%)
T. abdominalis	0	0	23	0	23	0	23	0	23	0
T. atratus	0	0	8	0	8	0	8	0	8	0
T. equalis	149	6(4.0%)	14	0	14	0	14	0	163	6(3.7%)
T. mularis	0	0	91	2(2.2%)	91	0	91	0	91	2(2.2%)
T. sparus milleri	0	0	145	4(2.8%)	145	0	145	0	145	4(2.8%)
T. lineola & *T. subsimilis*	0	0	210	5(2.4%)	210	0	210	0	210	5(2.4%)
T. sulcifrons	0	0	85	0	85	0	85	0	85	0
T. trimaculatus	5	2(40.0%)	30	4(13.3%)	30	1(3.3%)	30	1(3.3%)	35	6(17.1%)
T. wilsoni	0	0	68	0	68	0	68	0	68	0
Total	177	12(6.8%)	1033	38(3.7%)	1033	4(0.4%)	1033	3(0.3%)	1210	53(4.4%)

was 3.7%, and trypanosomes were cultured from the posterior gut and salivary glands. The infectivity rate of T. mularis was 2.2%, and trypanosomes were cultured from the posterior gut. The infectivity rate of the T. lineola-T. subsimilis complex was 2.4%, and trypanosomes were cultured from the posterior gut. No trypanosomes were cultured from T. sulcifrons or T. wilsoni.

Discussion

The diversity of Tabanidae in Cherokee County appeared to be higher than in Payne County. Wright et al. (1984) collected 23 tabanid species with malaise traps in Payne County through four years of tabanid collections as compared to 33 species collected in this study. However, the predominant species appear to be similar, and the five most numerous species were the same in both counties, if the T. lineola-T. subsimilis complex of Cherokee County are counted as T. subsimilis which appears to be the more common of the two species.

Tabanus lineola occurred in substantial numbers in Cherokee County. However, Pechuman (personal communication) speculated that it is a recent emigrant to the state and noted that considerable hybridization is apparently taking place between it and T. subsimilis. The T. stygius collected establish a southward extension of this species range in Oklahoma. Cherokee County is only the third Oklahoma county from which T. wilsoni had been verified (Pechuman, personal communication), and it appears to be a common tabanid species there.

Trypanosoma theileri-like organisms appear to be common in tabanids in Cherokee County; but the prevalence was much lower than

that reported by Krinsky and Pechuman (1975) for New York tabanids. This study confirms the hindgut is the most common organ to harbor trypanosomes in tabanids. However, the demonstration of positive cultures from the salivary glands of three flies of three different species raises the possibility that the trypanosome might be inoculated into the mammalian host with the bite. Also, the demonstration of positive cultures of the mouthparts of three flies after a period of 12+ hours raises the possibility that mechanical transmission might be a means by which T. theileri-like organisms are transmitted to the mammalian host.

CHAPTER V

THE USE OF FIAX® SEROLOGY FOR QUANTITATION OF *TRYPANOSOMA THEILERI* LAVERAN IN TABANIDAE (DIPTERA)

Introduction

Trypanosoma theileri Laveran is a cosmopolitan parasite of domestic cattle (Hoare, 1972), and it is generally considered non-pathogenic. However, several authors have associated it with various pathological conditions which include lymphocytosis (Cross et al., 1968), reduced milk production (Ristic and Trager, 1958) and abortion (Dikmans et al., 1957; Levine et al., 1956; and Kingston et al., 1982). Since Nöller (1916) first recovered trypanosomes that were indistinguishable from culture forms of *T. theileri* from horse flies in Germany, numerous workers have reported *T. theileri*-like organisms from various tabanid species. *Trypanosoma theileri* apparently undergoes cyclical development in the tabanid alimentary tract and multiplies there (Hoare, 1972). However, *T. theileri* quantities and infection longevity in tabanids have not been published.

This study was designed to describe the replication and longevity of *T. theileri* infections in tabanids using FIAX® (Fluorescent Immuno Assay, International Diagnostic Technology [IDT], Santa Clara, CA) serology which provides a method for quickly quantitating fluorescent

antibody reactions. The method used in this study was a FIAX competition test which is briefly described, with illustrations, in the Appendix. The FIAX system consists of a FIAX 100® fluorometer (IDT) and a key shaped plastic StiQ® (Surface Technique for Immunoquantification, IDT) with a disk made of cellulose acetate-nitrate polymer attached to either side of the distal end. The polymer is capable of binding proteins absorbed onto it.

Materials and Methods

Trypanosome Cultures

Trypanosoma theileri used as antigen to immunize rabbits and for FIAX procedures were continuously cultivated at 37° C in fetal bovine cell cultures using a modification of the method described by McHolland-Raymond et al. (1978) for Fetal Bovine-4-Bone Marrow cell cultures. Medium 199 with Hanks' Balanced Salt Solution (Grand Island Biological Co. [GIBCO]) supplemented with 0.5% bacto-peptone (Difco Laboratories), 0.2 µg/ml vitamin B_{12} (Med Tech, Inc.) and 10.0% heat inactivated fetal bovine serum (GIBCO). A mixture of antibiotics that included 25 µg/ml streptomycin (Pfizer Laboratories), 20 µg/ml gentamycin (Schering Corp.) and 50 µg/ml kanamycin (Bristol Laboratories) was also added to the culture medium. Culture pH was maintained between 6.5 and 7.5 with 10.0% $NaHCO_3$.

Fetal bovine spleen cells were obtained from the Oklahoma Animal Disease Diagnostic Laboratory and transferred to 75 cm^2 (200 ml) plastic culture flasks. When the monolayer became 75-95% complete the culture medium was changed and T. theileri inoculated onto it.

The original inoculum was heparanized blood from an experimentally infected adult cow. The blood was transferred to 16 X 125 mm glass culture tubes containing 10 ml of Veal Infusion Medium (VIM). When T. theileri became abundant (7 days) a few drops of the VIM culture were transferred to cell cultures. Trypanosomes were harvested from cell cultures at 1 or 2 day intervals and processed into antigens for rabbit immunization and FIAX reagent, or were frozen alive in 10% glycerin at -60^{o} C to provide a reserve supply of culture material.

Infection of Calves

Various breeds of male dairy calves, less than two months old, were infected with T. theileri and then exposed to tabanids. Calves were injected directly into the jugular vein with infected blood. The original inoculum was 3 ml of blood collected in an EDTA treated vacuum tube from a naturally infected calf admitted to the Oklahoma State University School of Veterinary Medicine Large Animal Clinic. Infections were maintained in experimental animals by the transfer of 50 ml of blood from infected calves to uninfected calves. Blood transfers were made 14 days after parasites were first observed in bloom smears from an infected animal.

Parasitemias were monitored in calves by daily collecting 10 ml of blood from the jugular vein into vacuum tubes treated with EDTA to prevent clotting. Blood was drawn into microhematocrit tubes and centrifuged. A microhematocrit tube was then scored and broken 1 mm below the buffy coat, and all of the buffy coat plus 1 mm of plasma were expressed onto a microscope slide, a coverslip added, and

observed at 100X under a microscope for swimming trypanosomes. This procedure was followed until circulating trypanosomes were no longer apparent.

Tabanid Exposure to Trypanosomes

To get tabanids to feed on an infected calf, flies were allowed to land on an adult cow where they were collected in clear plastic cups before they began to feed. Cups containing flies were then immediately transferred to the infected calf and held until the flies completed a blood meal. Cups were conical in shape and measured 50 mm high with a bottom end diameter of 30 mm and open end diameter of 44 mm. A collection cup was held in place by fitting over it a second cup which was attached to calf body hairs by spring clips tied to a rubberband passed through a pair of holes on the bottom of the second cup (Hollander and Wright, 1980b).

Fully engorged tabanids were removed by sliding a cardboard lid between the collection cup and calf. Flies were then placed in a chilled ice chest for transport to the laboratory where they were held for various periods of time. In the laboratory, tabanids were maintained in 950 ml cardboard cans fitted with cloth mesh tops. Ten or fewer flies were placed in a single can and were provided daily with water and 10% sugar solution soaked into cottonballs which were placed on the can tops. Flies were maintained at 24^{o} C $\pm$ 3^{o} with a 12 hour light/dark cycle. After being held in the laboratory for predetermined periods of time, tabanids were frozen at -60^{o} C until they were processed for FIAX testing. A large number of unfed tabanids collected in malaise traps from Payne County and Cherokee

County were also frozen. Nonfed flies from Payne County were used as negative-control flies to calibrate FIAX tests. Those from Cherokee County were collected in an attempt to identify the flagellates in a tabanid population from an area previously shown to be endemic for T. theileri-like organisms.

Experimental tabanids were prepared for FIAX testing by thawing them, removing the head, and making an incision that extended along the ventral side from the anterior prothorax through the anal opening. Entire alimentary tracts were then removed and placed individually in 15 X 200 mm disposable plastic tubes containing 1.5 ml of distilled water. Tubes with alimentary tracts were sealed and frozen at -20° C until they were used for FIAX analysis.

Trypanosome Ag Preparation

Trypanosoma theileri to be used as antigen (Ag) for FIAX tests were counted with a hemacytometer at the time they were harvested from cell cultures. They were then centrifuged for 10 minutes at 10,000 rpm and the culture medium decanted. The pellet was then resuspended in phosphate-buffered physiological saline (PBS), centrifuged for 10 minutes at 10,000 rpm and the PBS decanted. This was repeated for a total of three washes in PBS to remove all excess culture medium. The washed pellet was suspended in 1.5 ml of distilled water, freeze/thawed three times, homogenized with a Polytron set at 14,000 rpm for three minutes and frozen at -20° C until needed.

Control Serum Preparation

Two rabbits were immunized with T. theileri antigens. One rabbit was injected intraperitoneally with triple washed live trypanosomes in PBS. The rabbit was given three weekly injections and a fourth was given after another two weeks. One week after the last injection 35 ml of blood was taken from an ear vein, and serum was separated. This rabbit was maintained until it became seriously ill approximately two months after blood was first harvested. At that time, the rabbit was exsanguinated via heart puncture and the serum harvested.

A second rabbit was immunized with a series of subcutaneous injections of homogenzied T. theileri Ag using a similar injection schedule as was used on the first rabbit. One week after the last injection 35 ml of blood was taken from an ear vein and serum was separated from it. This was repeated one week later. Serum was frozen at -20^{o} C in 2 ml aliquots until needed.

FIAX Procedures

A total of 1.5 ml of packed T. theileri organisms were used as Ag to spot StiQs. These were washed three times in PBS, suspended in 1.5 ml of distilled water, freeze/thawed three times and homogenized with a Polytron set at 14,000 rpm for three minutes. The homogenized Ag was diluted 1:10 in 0.15% Tween-20 (Atlas Chemical Co.) in PBS, divided into 2 ml portions and frozen at -20^{o} C until needed. The conjugate was a 1:200 dilution of goat anti-rabbit IgG labeled with fluorescein isothiocyanate (Cappell Laboratories). A solution of 0.15% Tween-20 in PBS was used to wash unbound reagents

from StiQs.

Two types of FIAX methods were evaluated. A competitive inhibition test was conducted according to the following procedures:

1. StiQs were spotted with 25 µl of a 1:10 dilution of previously titrated Ag and allowed to dry.
2. A dilution series of quantified homogenated competing T. theileri antigen (CAg) was reacted in 1.5 ml of distilled water containing 25 µl of Tween-20 with control serum. Control serum and CAg were reacted for 30 minutes with agitation on a shaker. This step was done with various concentrations of control serum (usually 1:75), and done both with and without the addition of nonfed tabanid alimentary tract homogenates to the reactants. It was done without alimentary tracts to determine optimum control serum concentration and to establish baseline results for comparison when alimentary tract homogenates were included. Nonfed tabanid alimentary tract homogenates were added to the reactants to simulate FIAX testing of tabanids that had fed on infected calves so that the test could be calibrated. In those tests with alimentary tracts, the needed number of negative control fly alimentary tract homogenates were thawed, combined and thoroughly mixed (i.e. if 10 dilutions were to be tested, 10 alimentary tracts were thawed and combined). These mixtures were dispensed evenly in 1.5 ml portions into test tubes.
3. After the CAg competition step, the mixtures were centriguged at 10,000 rpm for 10 minutes or were filtered through Sera-Separa Filters® (Evergreen Scientific) to remove particulate material. The supernatents were then split into two 0.5 ml portions and

placed in 5 ml glass test tubes. StiQs with Ag were reacted with the supernatent in tubes for 10 minutes with agitation on a shaker. This procedure resulted in two replicates per sample (0.5 flies each).

4. Following the StiQ competition step, StiQs were transferred to 0.6 ml of buffered wash for 10 minutes with agitation to remove unbound Ab.

5. The StiQs were then transferred to tubes with 0.5 ml of anti-rabbit conjugate (1:200) and allowed to react for 15 minutes with agitation.

6. The StiQs were next placed in a second 0.5 ml buffered wash for 10 minutes and agitated on a shaker to remove unbound conjugate.

7. After the last wash, StiQs were read on the fluorometer and the fluorescence (FS) values recorded and plotted.

The cross-reactivity of the test Ab with related, non-target organisms was measured by substituting culture forms of other species for T. theileri in Step 2 of the competition test. Other organisms used in cross-reaction tests were Trypanosoma cervi Kingston and Morton, T. cruzi Chagas, T. musculi Kendall and an Oklahoma isolate of an undetermined Leishmania species. The resulting FS values were compared with those for T. theileri as an indication of cross-reactivity.

A second test designed to directly quantify T. theileri Ag was conducted according to the following procedures:

1. StiQs were spotted with 25 µl of various dilutions of the competing T. theileri (CAg) and allowed to dry.

2. StiQs with CAg were added to 0.5 ml of 1:101 dilutions of

serum in 0.15% Tween-20 and PBS for 20 minutes.

3. The remaining steps were run as in Steps 4-7 of the competition test.

Results

An evaluation of cross-reactivity of the T. theileri-specific antiserum indicated that little or no anti-T. theileri Ab bound with T. cruzi, T. musculi or the Leishmania sp. antigens. However, there did appear to be a cross-reaction with the closely related T. cervi (Table VII).

The FS values obtained from two preliminary FIAX competition tests using a series of two-fold dilutions of CAg were plotted. Regression analyses showed good correlations between FS values and the number of competing T. theileri organisms (Figure 8). However, when competition steps were run in the presence of homogenized tabanid alimentary tracts, correlations could not be made because of high background fluorescence. Addition of 25 µl of concentrated Tween-20 to the reagents plus filtration with Sera-Separa Filters following Step 2 reduced background fluorescence to an acceptable level. This procedure was used in all subsequent competition tests.

When results from several subsequent tests were plotted, regression analyses generally showed poor correlations between FS values and the number of competing organisms. When fly alimentary tract homogenates were added to Step 1, there was a tendency for increased replicate variability and for elevated FS values.

Because the alteration of test serum concentrations did not result in useful FS values, the ability of test Ab to bind with both

TABLE VII

A SEROLOGICAL COMPARISON OF TRYPANOSOMA THEILERI WITH FOUR RELATED ORGANISMS USING FIAX® SEROLOGY

Competing Antigen	FS Value
Test #1 (Particulate Ag)	
Trypanosoma theileri	99
T. cruzi	119
Leishmania sp. [1]	118
No Competing Ag	120
Test #2 (Whole Ag)	
T. theileri	95
T. cervi	88
T. musculi	127
No Competing Ag	128

[1] Oklahoma canine isolate (OKD), species uncertain.

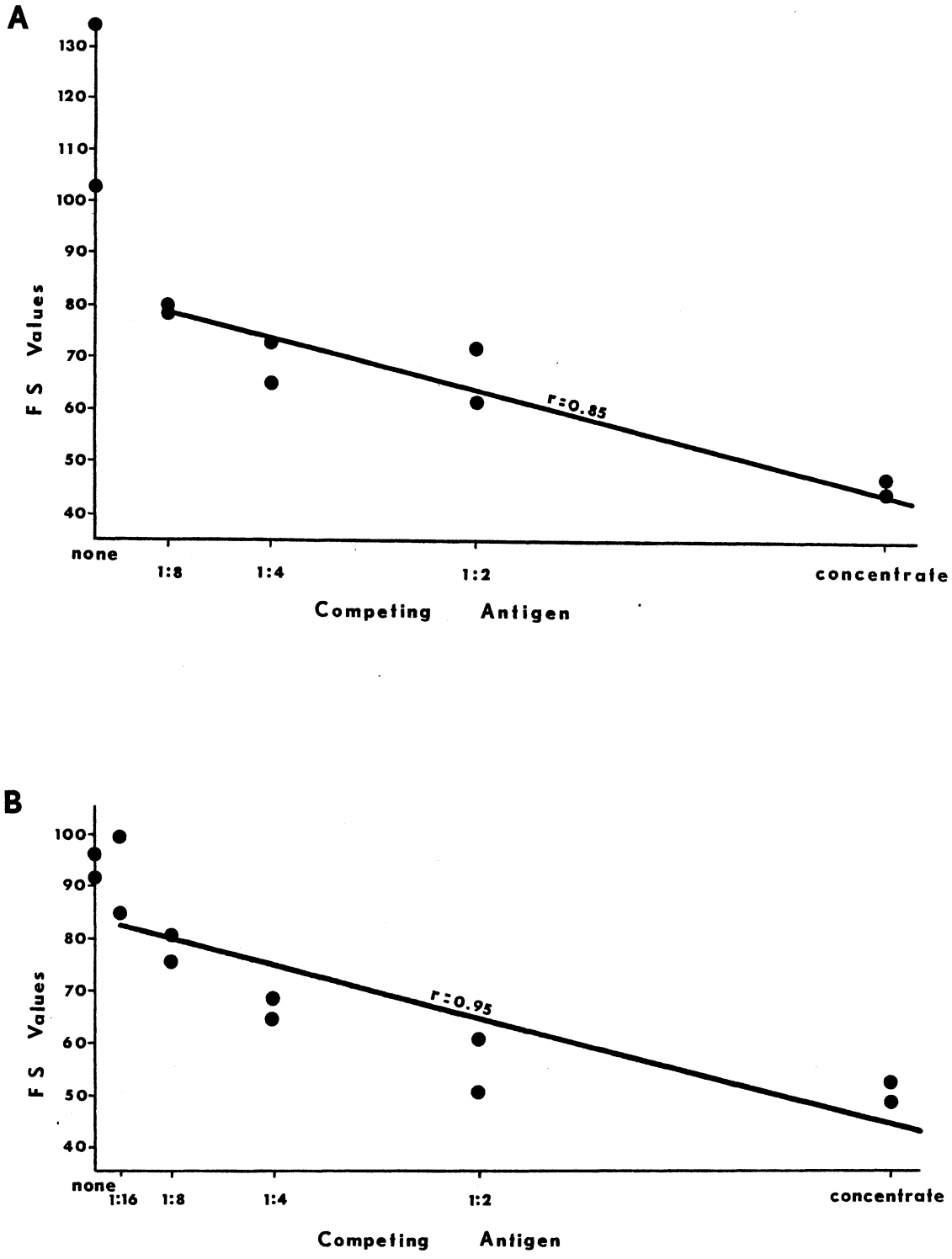

Figure 8. Regression Curves Showing the Correlation Between FS Values and Competing Antigen Concentration Using FIAX® Immunofluorescence Test for Trypanosoma theileri Quantitation.
A. Serum Dilution = 2:75
B. Serum Dilution = 1:75

T. theileri Ag and with goat anti-rabbit conjugate was then evaluated in an additional series of tests. The capacity of the T. theileri-specific antibody to bind CAg was tested by comparing the FS values obtained from a competition test using a standard amount of CAg and four test serum dilutions with FS values obtained from a test using the same series dilutions, but with no CAg. The test serum dilutions ranged from 1:52.5 through 1:211, and the amount of CAg used was equivalent to 969,770 organisms. Results indicated the CAg sufficiently bound to anti-T. theileri Ab to reduce FS values.

The ability of test Ab to bind goat anti-rabbit conjugate was tested by spotting StiQs with 25 µl of six test serum dilutions and reacting them with the anti-rabbit conjugate. The FS values were compared with similarly treated StiQs spotted with six dilutions of normal (non-immunized) rabbit serum. Results indicated the test serum bound more conjugate than did the normal serum.

Preliminary tests with serum from a second rabbit produced even higher FS values than those from the first rabbit, indicating it had a higher Ab titer. Therefore, this serum was used in all subsequent tests. The first competition test with serum from this rabbit resulted in FS values to which a regression line could be plotted with a good correlation between FS values and numbers of competing T. theileri ($r = 0.88$). Five CAg dilutions ranged from 947 through 15,153 T. theileri organisms. Correlations between FS values and CAg were generally poor when larger amounts of CAg were used in subsequent tests. Also, the introduction of homogenized tabanid alimentary tracts in Step 2 resulted in increased replicate variability that made meaningful correlations impossible. A regularly observed phenomenon

was an unexpected early peak in FS values in which a low CAg dilution resulted in higher FS values than occurred with no CAg at all. The result was a curve similar to that shown in Figure 9.

A second type of FIAX test, designed to provide FS values directly proportional to various amounts of T. theileri antigens, was also evaluated. A series of CAg dilutions were absorbed to StiQs and the StiQs exposed to control Ab. Serum from the second rabbit only detected 242,000 or more T. theileri organisms.

A total of 229 tabanids comprising five species were allowed to complete blood meals on a calf experimentally infected with T. theileri. These flies were held in the laboratory for periods that ranged from less than six hours to 22 days before they were frozen at -60° C (Table VIII). Eighty T. abactor collected in Cherokee County and 105 collected in Payne County in malaise traps were also frozen. However, except for the T. abactor alimentary tract homogenates from Payne County that were used in attempts to calibrate competition tests, these tabanids were not tested with FIAX because neither of the tests evaluated could be sufficiently refined to be useful.

Discussion

The FIAX competition test used in this study was a modification of a standard FIAX immunofluorescent serodiagnostic test, and it appeared to have potential as a tool to quantify trypanosomes in tabanids. It was designed to provide a quantitative measure of an approximate number of T. theileri organisms in infected fly alimentary tracts. Initial tests demonstrated the ability of FIAX to measure

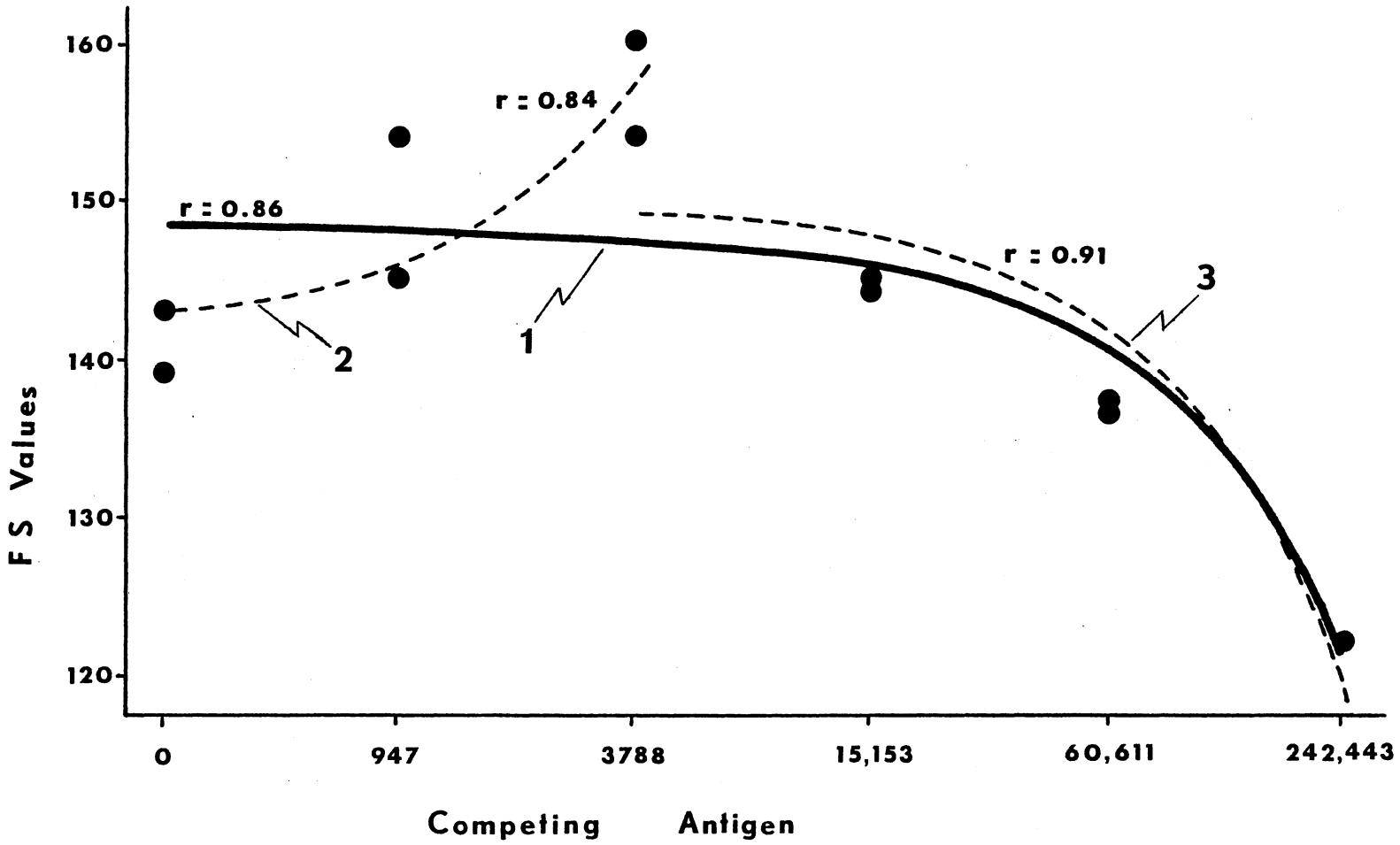

Figure 9. Non-logrithmic Representation of the Regression Curves for FS Values and Competing Antigen Concentrations with Correlation Coefficients for all Data Points (1) and for Data Points on Either Side of the Peak Response (2 & 3). FS Values Obtained Using a FIAX® Immunofluorescence Test for Trypanosoma theileri Quantitation.

TABLE VIII

NUMBER OF FIVE TABANID SPECIES ALLOWED TO BLOOD FEED ON A CALF EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA THEILERI* AND THE TIME THEY WERE HELD IN THE LABORATORY BEFORE THEY WERE FROZEN AT -60° C

Time Held	*Tabanus abactor*	*Tabanus equalis*	*Tabanus mularis*	*Tabanus venustus*	*Chrysops pikei*
< 6 Hours		7			
1 Day	5				
2 Days	2	6	11		
3 Days	20	14	12	2	
4 Days	5	9	5	9	1
5 Days	6	11	3	3	1
6 Days	15	8	10	2	
7 Days	8	2		2	
8 Days	8	3			
9 Days		7			
10 Days	5	2		3	
11 Days		4			
13 Days		3			
14 Days		1			
15 Days		5			
16 Days	4	2		1	
18 Days		1			
22 Days		1			
Total	78	86	41	22	2

varying amounts of T. theileri antigens, and there was a good correlation between decreasing FS values and the amount of T. theileri competing antigens.

However, correlations were generally poor in subsequent tests, because variations in FS values for sample replicates within tests were often greater than the variations between the treatment means. The addition of alimentary tract homogenates tended to increase sample variation even more and to unexplainably elevate FS values. These problems were not controlled by either filtration or centrifugation of particulates from samples prior to the addition of StiQs. Replicate variability might have been reduced with more accurate dispensing of reagents. The use of fresh CAg continually prepared from live T. theileri organisms might also reduce replicate variability. It was recently observed that prolonged storage of Ag prepared from Leishmania sp. (related organisms) at subfreezing temperatures affected their antigenecity (Dr. J. Carl Fox, personal communication). This may also have been true for Ag prepared from T. theileri.

The unexpected peak in FS values that occurred (Figure 9) may have been the result of saturated complexes of CAg and Ab binding to the StiQ. The premature peak in FS values would result in a false representation of the number of competing organisms. Consequently, this concentration phenomenon prevented extrapolation of accurate estimates of T. theileri numbers when there were fewer organisms present than those that resulted at peak fluorescence.

The FIAX methods evaluated in this study did not prove useful for quantifying T. theileri in tabanids. It is likely the number of organisms in at least a portion of tabanids would be lower than

the numbers that could be accurately determined by the FIAX methods used.

A test modification that might overcome the problem of a premature peak might be to add a standardized amount of CAg to all samples prior to the addition of control serum and samples in Step 2 of the competition test so that all extrapolations could be made from the excess antigen side of the peak. The amount of CAg added would have to be equal to or greater than the amount of Ag required to produce peak fluorescence, and FS values would represent the corresponding number of organisms less the standardized amount of CAg added. However, this would require larger numbers of organisms to be cultured than was possible in this study.

A similar FIAX competition test used to measure low levels of gentamicin in human serum (Tsay et al., 1980) could possibly be adapted to detect trypanosomes in tabanids. This test would require that T. theileri antigens be conjugated with a fluorescent dye and that anti-T. theileri antiserum be absorbed onto the StiQ sampler. Labeled competing organisms and unlabeled (sample) organisms in the tabanids would compete for the Ab on the StiQs, and FS values would be inversely proportional to the number of organisms in the fly.

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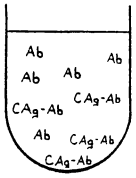
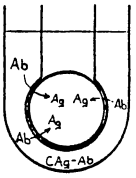
APPENDIX

A REPRESENTATION OF A FIAX® COMPETITION TEST USING HOMOGENATED TRYPANOSOMA THEILERI AS THE TEST ANTIGEN

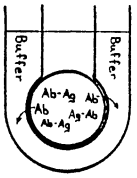
Step 1. Competing antigen (CAg) is added to a serum dilution and allowed to react. Antibody (Ab) specific for CAg is bound and rendered unavailable for subsequent steps.



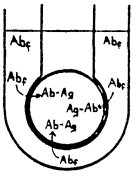
Step 2. A StiQ® with similar antigen (Ag) is added to the test solution to bind residual Ab.



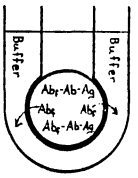
Step 3. The StiQ is transferred to a buffered wash solution to remove unbound Ab.



Step 4. The StiQ is transferred to anti-rabbit fluorescein-conjugated antiserum (Ab_f) which binds with any bound Ab on the StiQ.



Step 5. The StiQ is transferred to a second wash to remove unbound Ab_f.



Step 6. The StiQ is read on a FIAX 100® fluorometer. The resulting fluorescent (FS) value is a measure of Ab_f on the StiQ and is inversely proportional to the amount of CAg added in Step 1.

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Candidate for the Degree of

Doctor of Philosophy

Thesis: THE TABANIDAE ASSOCIATED WITH WHITE-TAILED DEER IN DIFFERENT HABITATS OF OKLAHOMA AND THE PREVALENCE OF TRYPANOSOMES RESEMBLING TRYPANOSOME THEILERI LAVERAN IN SELECTED SPECIES

Major Field: Entomology

Biographical:

Personal Data: Born in Okarche, Oklahoma, March 25, 1952, the son of Mr. and Mrs. R. H. Whittle.

Education: Graduated from El Reno High School, El Reno, Oklahoma in May 1970; received Bachelor of Science degree with a major in Wildlife Ecology from Oklahoma State University in May, 1975; received Master of Science degree in Wildlife Ecology from Oklahoma State University in December 1979; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in May 1984.

Professional Experience: Graduate Teaching Assistant, Department of Biological Sciences, Oklahoma State University, 1975-1978; Field Technician, Stream Alteration Research Project, Department of Biological Sciences, Oklahoma State University, 1976-1977; Employee, Oklahoma State University Museum of Natural History, 1977; Graduate Teaching Assistant, Department of Entomology, Oklahoma State University, Fall and Spring, 1978-1983; Graduate Research Assistant, Summer, 1980-1983 and Spring, 1984.

Societies: American Society of Mammalogists, National Wildlife Federation, Entomological Society of America.