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Bureau of Animal Industry

Preliminary Experiments

IN THE

Transmission of Anaplasmosis by Horseflies

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PRELIMINARY EXPERIMENTS IN THE TRANSMISSION OF ANAPLASMOSIS BY HORSEFLIES

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Introduction

Anaplasmosis in cattle has been under investigation during the past four years at the Oklahoma Agricultural Experiment Station in cooperation with the Federal Bureau of Animal Industry. During this period, the Entomological Department has collected a splendid representation of ticks found in Oklahoma, some 18 or 20 species, also many specimens of horseflies found in this locality. The distribution, identification, life history, and habits of these ticks and flies constitute a major part of this investigation.

Workers in the United States and other lands have incriminated various species of ticks as carriers of anaplasmosis, but any record of successful transmission with flies in foreign countries is unknown to the writers.

A study of field conditions, the prevalence of Tabanids, their occurrence, habits, and distribution has been found to correspond closely with the incidence of anaplasmosis. This is particularly true in swampy regions near ponds, inundated areas along rivers, lowlands of creeks, and in wooded areas. These are the places where horseflies breed and develop, and where the greatest annoyance to livestock occurs.

The biological manner in which these flies transmit the microparasite of anaplasmosis from infested cattle to healthy ones is unknown. The length of time infested flies remain potential carriers of the parasite under varying conditions is also a matter for further investigation. At present, it appears that inoculation of cattle may be accomplished directly by flies feeding on animals infested with the disease, and then immediately feeding on susceptible animals. At the present stage of our in-

[1]This is a cooperative project conducted by the Oklahoma Agricultural Experiment Station and the Bureau of Animal Industry of the United States Department of Agriculture. The personal services of one of the authors, Dr. George W. Stiles Jr., associate bacteriologist, and funds have been furnished by the above mentioned bureau.

vestigation, we do not know whether incubation of the protozoa occurs in flies, similar to malarial development in mosquitoes, or not.

Evidence is available which indicates that mechanical transmission of anaplasmosis is increasing on account of a lack of proper asepsis. Bull tongs, needles, tools used for dehorning, castrating and other surgical instruments in general that are brought in contact with the blood of afflicted and healthy animals in surgical operations are well recognized in the category of anaplasmosis transmission.

In view of such experiences by others, adequate measures have been instituted in our experimental work by using only individual sterilized needles for obtaining blood smears, and properly cleansed instruments for all surgical operations, even though of a minor character, in connection with our investigations of anaplasmosis.

This paper is not offered as a final publication by the authors on this project. The data presented, however, are considered as an important contribution of progress.

Previous Transmission by Flies

During the season of 1930, the authors[2] reported a single fatal case of experimental anaplasmosis. This case was known as cow no. 21. This cow was subjected to three species of horseflies. These include *T. gracilis* Wied., *T. sulcifrons* Macq., and *Chrysops sequax* Will. Each species fed on the cow 29, 7, and 5 times respectively after first feeding on a carrier known as no. 4, making a total of 41 bites on cow no. 21. The feeding was done between Aug. 19 and 25 inclusive.

Cow no. 21 died Dec. 17, 1930, 123 days after the first fly bite of Aug. 19, and 117 days following the last fly bite on Aug. 25. Definite microparasites were observed in her blood on Dec. 9, or 106 days after the last fly bite. Her first temperature rise was noted on Dec. 10.

The flies used in this experiment were caused to feed on a "carrier" animal, the blood of which often failed to show parasites when examined microscopically. The blood of this carrier never failed to produce anaplasmosis in susceptible cows when injected in quantities of from 7 to 40 c. c. The authors believe the long period of incubation can be explained by the small number of parasites in the carrier's blood and the comparatively few fly bites to which the cow was subjected in the experiment.

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[2]Oklahoma Agri. Exp. Sta. Rpt. 1926-1930, pp. 254-264, figs 2.

After having continued this investigation more than one year by using these and other species of flies as vectors, all evidence developed indicates that these findings were absolute.

Encouraged by the results of 1930 further fly experiments were planned for the summer season of 1931, consequently with the advent of warm weather and the reappearance of flies, additional experimental feedings began.

Collection of Flies

All of the flies used in this series of four experiments were collected along Cabin Creek lowlands, 3 miles west of Cushing and about 23 miles southeast of the Oklahoma A. and M. College and Experiment Station. No cases of anaplasmosis have ever been known in this immediate vicinity. This area contains a number of ponds, creeks, and timbered lands suitable for the breeding and sheltering of horseflies.

A Holstein bull served as bait for attracting the flies from which they were readily captured uninjured by means of small, ventilated pasteboard boxes. Each individual fly was retained in one of these boxes for use as long as it lived.

From 50 to 100 specimens were usually secured during the active fly season within a period of one to four hours, and immediately brought to the laboratory. Without unnecessary delay, every fly was given opportunity to feed, first on a carrier or acute case, then on a supposedly susceptible cow.

Each fly was replaced into its respective box immediately following its feeding periods and kept for use until its death occurred. Meantime on subsequent days they were given opportunity to feed. The initial feeding usually took place on the day of capture; however, those that refused to feed the first day were given opportunity to feed later. Only those flies that fed both on the diseased animals and the susceptible critters the same day were recorded as "direct" or "immediate" bites. Those flies which fed first on the diseased animals and then on the well cows on subsequent days before possible feeding again on the diseased cows were recorded as "delayed" or "deferred" bites.

Source of Anaplasmosis Used

Two animals were utilized as sources of anaplasmosis on which flies were allowed to feed, viz. bull no. 4 which was raised in the anaplasmosis lots, and cow no. 19 which was purchased from the College Dairy Department.

On June 25, 1930, the cow, no. 17, was injected intravenously with 40 c. c. of whole blood from carrier no. 4. Typical clinical symptoms of anaplasmosis appeared in this cow from July 24 until July 31, after which she recovered.

A second inoculation with blood from carrier no. 4 was

made Feb. 12, 1931, when 38 c. c. of whole blood was injected into cow no. 48. This animal developed anaplasmosis one month later, thus proving animal no. 4 was still a carrier at this time.

On May 10, 1931, a third check was made on animal no. 4 by injecting 7 c. c. of whole blood intravenously into cow no. 19. At the time of injection, cow no. 19 showed 6,200,000 red cells per cubic m. m. and 65 per cent hemoglobin (Sahli scale). On June 17 the hemoglobin was 15 per cent and the red cell count was 1,400,000 per cubic m. m. Her temperature during the morning of June 15 was 102.2°F. At noon it was 104.6°F., and during the evening it was 104.5°F. Microparasites present in the blood of cow no. 19 together with her clinical symptoms proved that she had acute anaplasmosis in a mild stage at the time she was used as a source of infestation for flies from June 15 to June 22, 1931, inclusive on cows numbered 45, 64, and 65. This cow (no. 19) recovered after being sick about two weeks.

Care of Experimental Animals

The animals used in these experiments were adult cows secured from the College dairy herd, or locally from herds likewise known to be free from anaplasmosis. They were examined clinically and microscopically before being used. They were housed in fly-proof screened sheds during the experiments.

Temperature records were taken daily and frequent blood smears made for microscopic observations. Occasional blood counts and hemoglobin tests were also made.

Control animals were always kept under the same housing conditions as the experimental ones. No spontaneous case of anaplasmosis has developed in the experimental lot, nor has any trouble from this malady developed locally in town or college cows. During the season of 1931 not a single case of anaplasmosis contracted under farm conditions has been reported within a radius of 25 miles from our experimental sheds. These facts are presented as supportive evidence that no unexplainable cases of anaplasmosis have occurred among the animals under experimentation.

Experimental Cow No. 45

This cow was four years old, weighed 740 pounds and was purchased locally.

This cow was subjected to bites from the following species of horseflies: *Tabanus gracilis* Wied., *T. fuscicostatus* Hine, *T. sulcifrons* Macq., *T. venustus* O. S., and *Silvius pollinosa* Will. These flies were induced to feed on an acute case of anaplasmosis, cow no. 19, before feeding on cow no. 45. From June 13 to 27 inclusive, the latter was subjected to a total of 43 fly bites of which 25 were direct and 18 were delayed, as shown by Table I.

Table I.

Species of Fly	Date 1931 June	Direct Feedings		Deferred Feedings						Total Direct	Total Deferred	Grand Total Bites
		No. 19	No. 45	1st Day	2nd Day	3rd Day	4th Day	5th Day	7th Day			
T. gracilis	13	1				1						
	15	7	7									
	16	3	3	1								
	17			1								
	19						1					
	20	2	2									
	21			1								
	22	4	4		1							
	23			3		1						
	24				1		1					
	25							1				
	26						1					
	27								1			
										16	15	
T. fuscicostatus	15	2	2									
	16	1	1									
	17			1								
	20	2	2									
	21			1								
										5	2	
T. sulcifrons	15	2	2									
										2		
T. venustus	16	1	1									
										1		
S. pollinosa	22	1	1									
	23			1								
										1	1	
			25	9	2	2	3	1	1	25	18	43

Summary of Table I.

The feedings are classified as follows:

T. gracilis	Direct feedings	16		
	First day delayed feedings		6	
	Second day delayed feedings		2	
	Third day delayed feedings		2	
	Fourth day delayed feedings		3	
	Fifth day delayed feeding		1	
	Sixth day delayed feeding		1	
		16	15	31
T. fuscicostatus	Direct feedings	5		
	First day delayed feedings		2	
		5	2	7
T. sulcifrons	Direct feedings	2		
		2		2
T. venustus	Direct feeding	1		
		1		1
S. pollinosa	Direct feeding	1		
	First day delayed feeding		1	
		1	1	2
	Total direct	25		
	Total delayed		18	
	Total direct and delayed			43

Cow no. 45 was exposed to 25 direct and 18 delayed feedings, making a total of 43 fly bites from June 13 to 27 inclusive. Her temperature remained normal from the beginning of the experiment until July 21.

The following temperatures occurred from July 21 to July 25 inclusive:

		A. M.	Noon	P. M.
July	21	103.4°F.	105.0°F.	104.6°F.
July	22	100.7°F.	103.4°F.	105.6°F.
July	23	104.7°F.	106.2°F.	105.8°F.
July	24	101.0°F.	101.6°F.	101.6°F.
July	25	101.7°F.	102.7°F.	

Cow no. 45 died of anaplasmosis on the evening of July 25, 1931.

Microscopic examinations of this cow's blood showed parasites present on July 22; they were negative until July 18. She showed the first clinical symptoms of disease 38 days after the first exposure to flies, and 24 days after the last fly feedings.

This cow developed clinical symptoms of anemia, icterus, weakness, and prostration typical of anaplasmosis, and died 42 days following the first fly bites, or 28 days after the last fly exposure.

Experimental Cow No. 64

This cow was 12 years old and weighed about 900 pounds. She was purchased from a healthy herd and was in good physical condition. Both microscopic and clinical examinations indicated that she was free from disease.

Table II.

Species of Fly	Date 1931 June	Direct Feedings No. 19	Direct Feedings No. 64	1st Day	Deferred Feedings 2nd Day	Deferred Feedings 3rd Day	Deferred Feedings 4th Day	Total Direct	Total Deferred	Grand Total Bites
T. gracilis	16	25	25							
	17	5	5	3						
	18	10	10	3	1					
	19	12	12	2						
	20	4	4	1						
	21	1	1	1	1					
	22	3	3	1						
	23	1	1	2	1	1	1			
			61	13	3	1	1	61	18	79

Table III.

Species of Fly	Date 1931 June	Direct Feedings No. 19	Direct Feedings No. 65	Deferred Feedings 1st Day	Deferred Feedings 2nd Day	Total Direct	Total Deferred	Grand Total Bites
T. sulcifrons	16	6	6					
	17	3	3	5	1			
	18				2			
	19	4	4					
	22	3	3					
			16	5	3	16	8	24

From June 16 to 23, 1931, cow no. 64 was subjected to fly bites of one species only, viz., *Tabanus gracilis,* which had previously fed on an acute case, cow no. 19. She was exposed to 61 direct or immediate bites, and 18 delayed or deferred bites, making a total of 79 bites during a period of eight days, as shown by Table II.

Summary of Table II.

Direct bites	61		
First day delayed bites		13	
Second day delayed bites		3	
Third day delayed bite		1	
Fourth day delayed bite		1	
Total number of direct bites	61		
Total number of delayed bites		18	
Total bites			79

Cow no. 64 remained normal in temperature (not above 101.4°F.) until July 23, just one month after the last fly bite, or 38 days from the first direct bite when the temperature in the morning was 101.4°F., at noon 105.5°F. and in the evening 105.5°F.

Preceding the rise of temperature on July 22, positive marginal bodies in the blood were noted microscopically with negative results previously on the 18th. No blood examinations were made on the intervening dates.

This cow continued with an irregular temperature until July 31, and then gradually returned to normal. During the intervening time she exhibited typical symptoms of anemia, jaundice, weakness, and refused to eat. She recovered later and was considered by the authors as having had a mild case of anaplasmosis.

Experimental Cow No. 65

When purchased from a local herd, this cow appeared normal; however, irregular temperatures began 11 days following exposure to the first fly bites, and continued somewhat abnormal during the entire period of observation.

Cow no. 65 was exposed to a total of 24 bites inflicted by *T. sulcifrons.* This species of fly fed on animal no. 19, an acute case of anaplasmosis. Of these feedings 16 were direct, and 8 were delayed, as shown by Table III.

Summary of Table III.

Direct bites	16		
First day delayed bites		5	
Second day delayed bites		3	
Total number of direct bites	16		
Total number of delayed bites		8	
Total number of bites			24

These feedings began June 16 and ended June 22.

Microscopically, this animal was negative for microparasites until Aug. 26 when positive marginal bodies were observed.

At this time her temperature ranged from 103.6°F. on Aug. 21 to 105.6°F. on Aug. 27, with irregular periods of high readings until Sept. 25, after which her temperature appeared to be normal.

As reported later under notes on *T. sulcifrons,* great difficulty was experienced with these flies in attempting to cause them to feed under captivity. Many hundreds of specimens were collected in order to secure the positive feedings recorded. During the time when marginal bodies were present on Aug. 26 and during the time of the high temperature readings, cow no. 65 showed icterus and anemia. Clinically speaking she had a mild attack of anaplasmosis. This was 66 days after the first fly bite, and 60 days following the last.

This cow originally gave a negative test for tuberculosis. No external abnormalities were apparent, except occasional difficulty in breathing.

On Sept. 16 another cow, no. 79, was injected subcutaneously with 20 c. c. of whole blood from this cow, no. 65. Twenty-four days later, Oct. 10, cow no. 79 developed anaplasmosis, thus proving cow no. 65 carried anaplasma at the time of the injection. No. 79 was treated with quinine per os and eventually recovered.

The unexplainable temperature observations on cow no. 65 make her an atypical case. However, such irregularities have been encountered in natural outbreaks of anaplasmosis, hence the authors deemed it advisable to record this experience for the benefit of future observers.

Experimental Cow No. 67

Cow no. 67 was purchased June 15, 1931, from a healthy herd, examined and held under observations until July 1, at which time she was first exposed to flies.

From July 1 to July 31 she was subjected to the bites of *T. venustus* which previously fed on a known carrier, animal no. 4. She received a total of 115 bites of which 47 were direct and 68 delayed, as shown by Table IV.

Summary of Table IV.

Direct bites	47		
First day delayed bites		20	
Second day delayed bites		13	
Third day delayed bites		11	
Fourth day delayed bites		8	
Fifth day delayed bites		6	
Sixth day delayed bites		4	
Seventh day delayed bites		3	
Eighth day delayed bite		1	
Ninth day delayed bite		1	
Eleventh day delayed bite		1	
Total number direct bites	47		
Total number delayed bites		68	
Total bites			115

Table IV.

Species of Fly	Date 1931 July	Dir. Feedings		Deferred Feedings										Total Direct	Total Deferred	Grand Total Bites
		No. 4	No. 67	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day	8th Day	9th Day	11th Day			
T. venustus	1	1	1													
	2	1	1													
	3	3	3													
	4	4	4													
	5	2	2													
	7	10	10													
	8	1	1													
	9	7	7													
	11	1	1													
	14	7	7													
	15	1	1													
	16	2	2													
	18	1	1													
	22	2	2													
	24	1	1													
	25	2	2													
	28	1	1													
	Aug.															
	2			1												
	4			1												
	5			2	1											
	6				1											
	7					1										
	8			2			1									
	9			1	1											
	10			1	1	2										
	12			1												
	13				1											
	14					1										
	15			5												
	16			1	3	1		1								
	17			3	1	2	2									
	18				2	1	2									
	19			1		2	1	2								
	20				1	1	2	1	1							
	21							2	1	1						
	22								1	1	1					
	27					1						1				
	30												1			
	31															
	23			1					1							
	24				1					1						
			47	20	13	12	8	6	4	3	1	1	1	47	68	115

Cow no. 67 showed two days of variable temperatures, viz. July 26 (101.6-102.6°F.) and Aug. 16 (102.0-103.0°F.) for one day periods each during the two months. Marginal bodies were first observed in this cow's blood on Sept. 1, and were reported as "suspicious." Later, on Sept. 3, they were found to be positively present. Temperature readings remained normal until 8 days later when on Sept. 11 the records show a. m. temperature 101.8°F.; noon 104.7°F.; and p. m. 104.8°F. Abnormally high temperatures prevailed until Sept. 22, when a return to normal was noted with an uneventful recovery.

This cow was treated with half-ounce doses of quinine per os twice daily beginning with the first appearance of symptoms during her febrile period.

Dating from the beginning of feeding July 1 until the temperature rise on Sept. 11, the number of days of incubation was 73; however, if counted from the last fly feeding date on July 31, the incubation period was 42 days. When the incubation period of this case is compared with those of cows numbered 45 and 64, the following variations were observed:

Cow no. 45—longest period 36 days, shortest 24 days
Cow no. 64—longest period 37 days, shortest 30 days
Cow no. 67—longest period 73 days, shortest 42 days

The length of the incubation period with cow no. 67 can probably be explained on the basis of using carrier animal no. 4 whose blood doubtless contained very few parasites as compared with the acute case, e. g., cow no. 19 used for cows nos. 45 and 64.

Summary

(1) The net result of the season's experiments comprises four cases of experimental transmission of anaplasmosis from infested cattle to healthy animals.

(2) Experimental cow no. 45 was exposed to a total of 43 fly bites composed of 25 direct and 18 delayed feedings. These bites were from five species of flies: *T. gracilis, T. sulcifrons, T. venustus, T. fuscicostatus, and S. pollinosa.* The two latter species may or may not have been active as vectors, since experiments were not conducted later to verify them as such. She developed anaplasmosis 38 days after the first fly bites, and 24 days following the last fly feedings. These flies were fed on an acute case. This cow died of anaplasmosis 42 days after the first fly bites and 28 days following the last fly bites.

(3) Experimental cow no. 64—infested by the bites of one species of horse fly only, *T. gracilis.* She was subjected to 61 direct bites and 18 delayed or a total of 79 bites during a period of 8 days. These flies fed on an acute case. This cow remained normal for exactly one month after the last fly bite, or 38 days

following the first fly bites, when she developed a mild case of anaplasmosis and recovered after being sick for about 10 days.

(4) Experimental cow no. 65. This animal was exposed to a total of 24 bites inflicted by *T. sulcifrons* only, 16 direct feedings and 8 delayed, over a period of 6 days. These flies were also fed on an acute case. She developed a very mild typical case of anaplasmosis 66 days following the first feeding, or 60 days after the last bite, and recovered after about one week's illness.

(5) Experimental cow no. 67. *T. venustus* was the only species of horse fly used to infest this cow. She received 47 direct bites and 68 delayed bites, or a total of 115. This animal showed typical clinical symptoms of anaplasmosis 42 days after the last fly bite or 73 days following the first exposure to flies. She recovered.

(6) It will be noted that the first three cows, numbered 45, 64, and 65, were exposed to flies which had fed on an acute case of anaplasmosis, no. 19, showing a rich infestation of anaplasma, while the fourth, or last case, i. e., cow no. 67, was exposed to flies fed on a carrier animal, no. 4, whose blood contained relatively few microparasites as compared with the acute case. This fact will probably explain the longer period of incubation in the latter case.

(7) The incidence of anaplasmosis in this territory corresponds to the greatest prevalence of horseflies, which occurs during the hot months of July, August, September and October. An occasional case of the disease may, however, be encountered during other months of the year.

APPENDIX

Habits of Flies Used

Our experience with the different species of flies used in these experiements[3] varied greatly in regard to their biting habits under captivity.

[3]Species according to the figure numbers were determined by specialists of the U. S. Dept. Agri., Bur. of Ent., as follows: Figures numbered 1 and 2, Dr. J. M. Aldrich; figures numbered 3 and 4, Dr. C. T. Greene; and *Tabanus fuscicostatus* by Dr. Alan Stone.

The manner in which they attacked animal life also varied; likewise the time of day they appeared as well as their seasonal occurence. In regard to their biting habits under captivity, some fed without hesitation, while others were induced to feed only with much patience and difficulty.

Tabanus sulcifrons

This species shown as fig. 1, was encountered most frequently in this locality biting horses, mules, cattle, and even swine. It appeared from June 1 to October 15. The period of greatest occurrence was during July or August. Many individuals refused to feed in confinement. Very few fed readily.

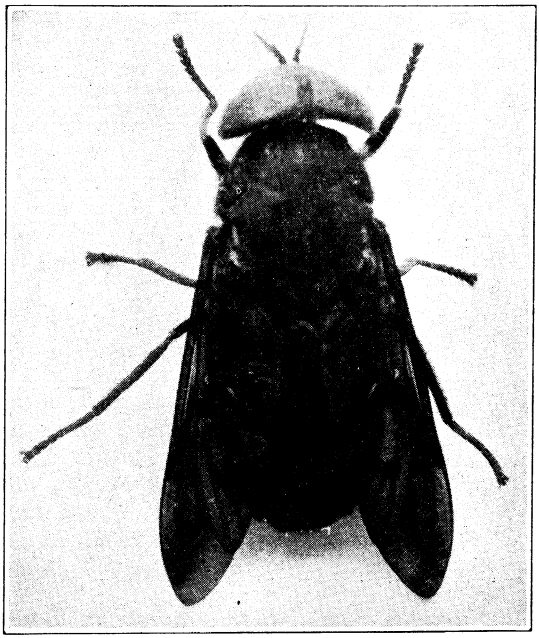

Fig. 1. *Tabanus sulcifrons* (X4).

The fly usually fed on the back and sides of the animals which it attacked. Many cattle bitten by this species showed raw, bleeding areas behind their withers where their tails or heads failed to dislodge the flies. This species was sometimes observed in large numbers perched in shaded places on trees, especially elm (*Ulmus americana*) in thickly wooded areas. They were individually located here and there on the trunk, main limbs, and smaller branches of the trees. As many as 50 could in some instances be observed on a single well branched tree of 12 to 15 feet in height. They were not exceptionally easily disturbed, although they could not be easily captured without the use of an insect net.

A rather remarkable peculiarity of this species of fly was observed by one of the authors (Sanborn) as follows:

"Swarming of the horsefly, *Tabanus sulcifrons* Macq. While sleeping on a front yard porch at Girard, Kansas, I was awakened before daylight at 4:30 a. m., July 15, 1930, by a tremendous buzzing, musical sound. It was somewhat similar to a distant tremulous exhaust from a locomotive high toned safety valve, or to the hum of a cream separator while in action. The buzz was coarser than that made by a swarm of bees in flight, and similar to and more comparable to drones than to worker bees.

"The air was entirely calm. There was little or no dew. Myriads of these flies were present from 5 to 15 feet from the ground (it was yet too dark to see well) near and in elm trees in the yard. These would remain buzzing in one place in the air for a few moments and then suddenly dart away a few feet distant and again poise and hover. This swarming continued until about 4:45 a. m. when it suddenly subsided. When broad open daylight came, practically all had disappeared except a very few specimens which had alighted on the limbs of the trees."

During the same day, Sanborn and Stiles continued to make observations and found many specimens of *sulcifrons* on the sidewalks in front of business house windows around the Girard city public square. The front of a hardware store with large plate glass front windows seemed to be a special death trap for them. It appeared that flies from a distance mistook the hardware in the store for trees and windows for water extending back into the store. Being attracted by this they would fly head-on against the plate glass with such force, as Mr. Walter Locke in the hardware store said, "to break their necks!"

Later on Aug. 22, 1931, Mr. Armine Maxwell, an advanced student doing insect survey work in northern Oklahoma for the A. and M. Department of Entomology, made the following statement: "On Thursday morning, August 20, 1931, about 5:00 o'clock a. m., at special request, I went to Mr. Dale Euriel's house just south of Medford, Oklahoma, to see about some kind of an insect that had been awakening him in the mornings. When I got there, I found it to be the commonly known horsefly, *Tabanus sulcifrons* Macq. His front yard was alive with them. Most of them were just a little higher than I could reach. They would remain in one place and buzz.

"On Aug. 27, 28, and 29, I went back to make similar observations, but due to the low temperature on the 27th and 28th the flies were inactive. On the 29th they came out at 5:20 a. m., and remained out until about 5:35 a. m. I collected a dozen, which were all males. I observed from the top of an elm tree a swarm of perhaps a dozen flies collected together into a ball about the size of a person's hands doubled together, and leave through a small opening between the limbs and leaves of the trees."

Mr. Maxwell also said that:

"Dr. R. H. Painter, Professor of Entomology at Kansas State College, states that on Aug. 1, 1921, he observed large numbers of Tabanids in pecan trees near Brownwood, Texas, at Pecan Bayou. He also stated that these flies made some noise and were mostly males. These flies were identified by Frank Hall as *Tabanus sulcifrons.*"

These observations seem to be the first of the kind recorded pertaining to *T. sulcifrons.* They doubtless pertain to mating habits of the species. Some observations of a similar nature have been made by Snyder[4] and by Mosier and Snyder[5] on *Tabanus americanus* Forst. and other species.

Snyder states, p. 125, that it is believed that the flies when hovering reverse and hover upside down with abdomen pointed upwards and legs sticking up. Later, Jones[6] described this same activity of the *T. americanus* as the dawn flight or mating flight.

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[4]Snyder, T. E. Proc. Ent. Soc. Wash., Vol. XIX, 1917 nos. 1-4, pp. 141-145, plates 2.
[5]Mosier, C. A. and Snyder, T. E. Vol. XX, no. 6, 1918, pp. 115-126.
[6]Jones, P. M.—Mss. Observations were similar to those of Snyder.

Tabanus gracilis

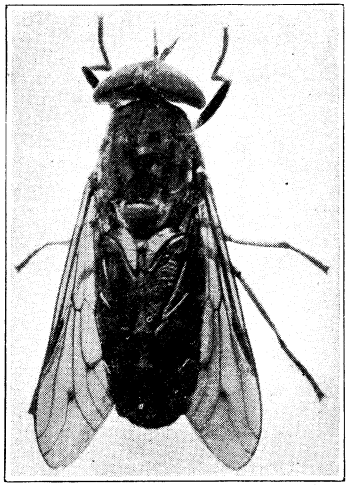

Fig. 2. *Tabanus gracilis* (X4).

This fly, shown as fig. 2, was the most vicious biting species of fly in our series of experiments. It is a grayish, medium sized fly. One of these flies bit continuously on a cow when undisturbed for a period of 10 minutes. For the most part, it was frequently seen to attack the legs, sides, and under parts of the animals. When engorging, it was loath to detach quickly.

A series of microscopic examinations of this species after engorging on a sick anaplasmosis cow showed marginal bodies in the undigested blood corpuscles within the fly's abdomen for a period of 30 hours after ingestion.

Tabanus venustus

The fly, shown as fig. 3, is a medium sized horse fly with brownish mottled wings. The prevalence and distribution of this species appears to be limited in this territory. Its first appearance noted was June 16 and its greatest occurrence was during July and August.

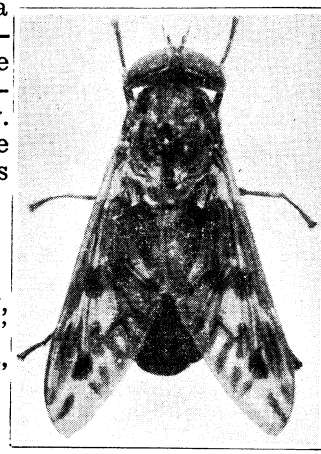

Fig. 3. *Tabanus venustus* (X4).

Tabanus fuscicostatus

This species is a small horse fly, commonly called a "green head." It was noted over rather a wide area, often attacking the head, ears, legs, and under parts of the animals. Specimens generally died soon after being captured.

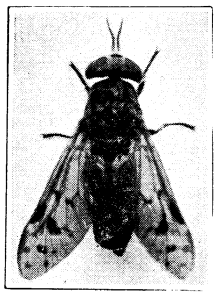

Fig. 4. *Siluius pollinosa* (X4).

Silvius pollinosa

This fly, shown as fig. 4, is a small grayish, speckled winged fly. It was found to be limited as to occurrence and distribution.