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THE TRANSMISSION OF ANAPLASMOSIS BY HORSEFLIES (TABANIDAE)

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PREFACE

Anaplasmosis or gall sickness in cattle causes serious losses to the livestock industry in some parts of Oklahoma every year. Because the fatality rate is high, preventive measures are of the utmost importance. The investigations reported upon in this bulletin have shown that several species of bloodsucking flies can, under certain conditions, spread the disease from sick and "carrier" animals to healthy animals. The conditions under which such infection can occur have been carefully investigated. These facts provide a basis for preventing the spread of the infection and for minimizing serious losses.

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THE TRANSMISSION OF ANAPLASMOSIS BY HORSEFLIES (TABANIDAE)

By D. E. Howell, C. E. Sanborn, L. E. Rozeboom, G. W. Stiles, and L. H. Moe

The first work on the transmission of anaplasmosis was done by Smith and Kilborne (*1*) in 1893 while they were investigating Texas cattle fever. They considered it a stage of *Piroplasma bigemina.* Theiler (*2*) in 1910 showed that the bodies seen by Smith and Kilborne were distinct disease entities which could be transmitted by *Boophilus decoloratus* (Koch), and in 1911 (*3*) by means of *Rhipicephalus simus* Koch he transferred anaplasmosis in a pure state, that is, free from Texas cattle fever. Since that time, at least 18 species of ticks have been incriminated as vectors, some congenitally from parent to offspring through the egg and others only from stage to stage.

Several authors (*4, 5, 6*) have shown the importance of mechanical transmission in the spread of anaplasmosis. Such cases may be called "man made" as they are due to carelessness in performing surgical operations, dehorning, tipping, vaccination, drawing blood and other processes that may convey infection from carrier to healthy animals unless adequate cleanliness of instruments and other aseptic precautions are observed. Due to anaplasmosis transmitted in this manner, widespread losses of valuable animals have been reported, often during cold weather when the natural vectors are inactive.

From a world standpoint, ticks probably constitute the most important transmission factor; but in some regions this may not be true. In the Oklahoma area some proven tick vectors are common during the winter and spring, but with the onset of hot, dry weather they become relatively inactive and are seldom found on cattle.

In this locality, cases of anaplasmosis which cannot be traced to mechanical transmission frequently occur during the warm summer months. Furthermore, the disease is usually associated with partially wooded areas where streams and ponds may be found. Sanborn et al. (7) when discussing these conditions made the following statement:

> A study of field conditions, the prevalence of tabanids, their occurrence, habits and distribution has been found to correspond closely to that 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.

That a similar condition exists in Kansas is indicated by the following quotation from the Kansas Agricultural Experiment Station report (*8*):

> Apparently there is a relationship between the presence and activity of Tabanidae and the disease anaplasmosis. These flies are noted to be more numerous during the seasonal prevalence of anaplasmosis. For instance, the large brown variety representing the greater number present are noted to appear about the latter part of June and continue their activity until the middle of September. Approximately thirty days after their appearance and activity are noted, the largest number of sick cattle appear. Within forty days after the departure of this species of Tabanidae, there are but few field cases of anaplasmosis.

An analysis of the collection data for the horseflies found in the Entomology Museum at the Oklahoma A. and M. College showed that the largest number of flies were taken during the latter part of July. The earliest collection date was April 14 *T. dodgei* Whitney), and the latest November 6 (*Tabanus giganteus* DeGeer and *T. sulcifrons* Macquart).

These epidemiological factors strongly suggested that anaplasmosis might be spread by horseflies, so a thorough examination of their importance as vectors was undertaken at this station.

In 1930, Sanborn et al. (*9*) reported a successful transmission under laboratory conditions of a single case of anaplasmosis by combined *Chrysops* and *Tabanus* flies. Two years later (*7*) the transmission of four additional cases was mentioned by these authors. Sanders (*10*) incriminated *T. fumipennis* Weid while working in Florida and Morris (*11*) in Louisiana reported a transmission of *T. atratus* Fab. by direct transfer.*

Despite this positive evidence, the ability of horseflies to transmit anaplasmosis has been questioned by several able workers. This viewpoint was expressed by P. J. Du Toit while speaking at the 12th International Veterinary Congress (*12*).

> All workers on this subject will agree with the eminent parasitologist Maurice Hall (1930) that there is at present absolutely no evidence based on carefully controlled experiments to substantiate the belief that biting flies are responsible for the spread of anaplasmosis.

In an effort to cast some light upon this important controversial question, this bulletin presents the results of the past eleven years work at the Oklahoma Agricultural Experiment Station on horsefly transmission of anaplasmosis. Forty-nine

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* Lotze and Yiengst report three positive mechanical transmissions by the horsefly *Tabanus sulcifrons* (Macquart). Amer. Jour. Vet. Res., Vol. 2, No. 4, pp. 323-326.

carefully controlled experiments are summarized. Fifteen of these experiments ended in the definite transmission of anaplasmosis from sick to healthy cows.

EXPERIMENTAL CONDITIONS

The first work with horseflies was done in small isolation sheds where the animals could be kept free from any other arthropods. These buildings were large enough to hold two cows. If flies were not available near the isolation sheds, the cows were loaded into a fly-tight trailer and taken to the flies. This procedure was open to criticism, so an elaborate isolation barn was built strictly for work on anaplasmosis. This barn contains eight separate isolation units, each capable of comfortably housing four animals. Sixteen-mesh screen wire covers all openings, and all doors are double with a trap hallway between to guard against the entrance or exit of arthropods. Space is provided inside the barn for the storage of a year's supply of food. No animal is allowed to leave the isolation barn and enter again and, so far as is known, they are kept free of any parasites except those experimentally introduced.

Flies used are captured in small pasteboard boxes from healthy cows in areas free from known anaplasmosis and kept in the boxes until ready for feeding.

Cattle are obtained from the college dairy herd if possible, as a complete history of each of these animals is obtainable. If cattle are not available from this source, animals are purchased at the semi-monthly community sale in Stillwater, kept in the isolation barn several weeks and checked by blood smears before use. Some of the cattle have been kept in the barn over two years, yet, except when experimentally transmitted, no cases of anaplasmosis have been noted.

EXPERIMENTAL PROCEDURE

The feeding of horseflies is accomplished by individually releasing the flies on the backs of cattle and confining them to a small area by covering with a 4- or 6-inch glass funnel. When interrupted feedings are desired, the fly is allowed to feed to half-repletion, as determined by the swelling of the abdomen, and then quickly transferred to the second cow. Transfer is accomplished by slipping a cardboard under the funnel to trap the fly in the funnel, and then releasing it again on the second animal to resume feeding. No bite is counted on the second animal unless definite feeding is observed. When delayed feedings are desired, the flies are returned to their boxes and kept until the next feeding period. Flies which escape fly to the window and can be easily recaptured. By regulating the temperature and humidity it is possible to keep flies alive a maximum of 20 days before refeeding. As many bites as possible are obtained during a single day, but the scarcity of flies frequently makes it necessary to extend the feeding period over several days.

Much variation between species is noted in the ease with which flies may be fed. *Tabanus abactor* Phillip and *T. erythraeus* (Bigot) are not easily disturbed, feed readily while confined under a funnel, and quickly resume feeding when interrupted or transferred. *Tabanus atratus Fab., T. sulcifrons* Macquart, *Silvius pollinosa* Will. and *Chrysops* sp? on the other hand are very easily disturbed, must be very hungry before feeding in captivity, and when placed on an animal under a funnel frequently batter themselves severely against the wall of the funnel. Immediate transfer feedings with these species are very difficult to obtain and require large numbers of flies.

The authors have had no success whatever in feeding flies by liberating them in an isolation stall, as the flies merely go to the window or ceiling and stay there without making any attempt to feed. Other authors (*8, 9*) report similar failures where this type of feeding has been attempted. It is possible that the laborious and time-consuming method used in the authors' experiments in obtaining the bites may explain the success in transferring anaplasmosis by horseflies. Probably the species of fly used is also very important, not because one fly is able to carry the disease more easily than another, but because one lends itself to experimental work while other flies are difficult to use.

The animals used to infect the horseflies are considered to be in the acute stage of anaplasmosis if more than one percent of the erythrocytes shows marginal bodies. A carrier animal is any animal which has had a definite case of anaplasmosis but no longer shows definite microscopic evidence or clinical symptoms of the disease.

These experiments have been classified into three groups for convenience during discussion: (1) immediate transfer feeding only, (2) immediate and deferred feedings, and (3) deferred feedings only, depending on the time elapsed between the bites on the sick cow and the healthy one. A further division based on the stage of the disease in the infecting cow has been used for each group.

(1) IMMEDIATE TRANSFER FEEDINGS ONLY

Flies Fed on Clinical Case

In an attempt to determine the number of fly bites necessary to transfer anaplasmosis, as well as the species of flies that can be used successfully, 12 experiments involving 6 species of flies have been performed.

Animals Nos. 74 and 241 each received a single *Tabanus abactor* bite. Neither animal subsequently showed marginal bodies. The next year No. 74 received 3 bites by *T. atratus* with negative results. *T. atratus* flies were allowed to bite 6 times on animal No. 80, but no anaplasmosis developed. Twelve bites by *T. sulcifrons* from August 16 to 18 failed to carry the disease to No. 5; but 13 bites by *T. oklahomensis* Stone caused the appearance of marginal bodies in the blood of animal No. 117, 45 days after the first fly fed. This represents the smallest number of fly bites from which transmission of anaplasmosis took place.

Between June 9 and July 14, animal No. 229 received 53 *Tabanus abactor* fly bites. These bites were obtained over a period of 36 days to determine the effect of delay between bites. Marginal bodies did not appear until the experimental inoculation with virulent blood.

Eighty-three *T. sulcifrons* bites, 8 from August 2 to 4, and 75 between August 15 to 23, produced a clinical case of anaplasmosis in animal No. 164, 61 days after the first bite.

A positive case was produced by another species of tabanid, *T. americanus,* in cow No. 115, 87 days after the first bite. This animal received 122 bites, all on August 19.

Animals Nos. 237 and 238 developed clinical cases of anaplasmosis as a result of 110 and 170 *Tabanus abactor* bites, respectively. All feedings on No. 237 were received July 17, and 22 days later blood smears were positive. Between July 25 and August 25 animal 238 received 170 bites. Thirty-two days after the first bite, the animal was positive.

Three hundred and forty bites over a period of 14 days by *T. erythraeus* failed to produce anaplasmosis in animal 158 but when the cow was inoculated later with virulent blood, it was impossible to demonstrate any marginal bodies or clinical symptoms. It seems therefore necessary to assume that this animal was a carrier and previously had an attack of anaplasmosis before being used in the fly experiment.

These experiments have been summarized in Table I.

Table I.—Immediate Transfer Feedings From Clinical Cases.

Number of cow	Dates of Feedings	Species of Fly Used	Total Number of Bites	Days from First Bite to Appearance of Anaplasmosis Bodies	Results	Susceptibility of Animal	Remarks
* 5	8/16-18	T. sulcifrons	12	---	Neg.	+	Infected next year by flies.
74	8/24	T. abactor	1	---	Neg.	0	No record of susceptibility check; kept four years and used in two more experiments.
74	5/29-6/9	T. atratus	3	---	Neg.	0	Same animal as above.
80	9/4	T. atratus	6	---	Neg.	+	Checked by blood inoculation.
115	8/19	T. americanus	122	87	Pos.	+	Died of anaplasmosis.
117	4/21-22	T. oklahomensis	13	45	Pos.	+	Used as carrier animal for later work.
158	7/20-8/4	T. erythraeus	340	---	Neg.	--	Inoculated intravenously with 10 cc virulent blood. Negative.
164	8/21-22	T. sulcifrons	83	61	Pos.	+	Recovered and used as carrier.
229	6/9-7/14	T. abactor	53	---	Neg.	+	Positive after blood inoculation.
237	7/17	T. abactor	110	22	Pos.	+	Recovered.
238	7/25-8/25	T. abactor	170	32	Pos.	+	Recovered.
241	9/11	T. abactor	1	---	Neg.	+	Positive after blood inoculation.

* Reported by Sanborn et al. 1930.

Flies Fed on Carrier Cases

Ten animals were used in direct transfer feedings involving flies which had fed only on carrier cases. Six species of the genus *Tabanus* were used individually, as well as two samples of *Chrysops* sp? and *Tabanus* sp?.

As may be seen in Table II, only one cow, No. 113, which was exposed to 7 *T. sulcifrons* August 1 and 79 August 22, developed anaplasmosis as a result of these experiments. Other cows received as few as 8, and as many as 824 bites, yet failed to sicken. It should be noted, however, that neither animal No. 74, which received 824 bites, nor No. 168, which received 140 bites, was shown to be susceptible. No. 168 was negative when inoculated with virulent blood; and, unfortunately, no test was made of the susceptibility of No. 74.

(2) IMMEDIATE AND DEFERRED TRANSFER FEEDINGS

Seventeen animals were used in a series of experiments designed to determine the effect of immediate and deferred transfer feedings. Tsetse flies are known to carry trypanosomes to normal animals for a few minutes mechanically and after several days biologically, so this series was set up to test the ability of horseflies to spread anaplasmosis in a like manner.

This type of test made it possible to use a smaller number of flies because they could be used twice rather than once, the first time for direct feeding and the second time in deferred feeding. Flies which received an infecting feed, yet refused to feed at once on the second cow, could be used the following day or later. Flies once infected were not given a second infective feed even though they refused to feed for several days. Ten species of flies in three genera, *Tabanus, Chrysops,* and *Silvius,* were used. Nine of the 17 animals obtained clinical cases of anaplasmosis as the result of the bites of these flies. Tables III and IV summarize the work.

Flies Fed on Acute Cases

Experiments with *Chrysops sp?* and *Silvius pollinosa* were negative even though a fairly large number of bites were obtained. During the period between June 12 and 22, 41 bites by several species of *Chrysops* were obtained on animal No. 18. Twenty-four of these were direct and 17 were deferred 24 or 48 hours. Results were negative even though the cow was susceptible.

Table II.—Immediate Transfer Feedings from Carrier Cases.

Number of cow	Dates of Feedings	Species of Fly Used	Total Number of Bites	Days from First Bite to Appearance of Anaplasmosis Bodies	Results	Susceptibility of Animal	Remarks
73	8/21-25	T. erythraeus	50	---	Neg.	+	
74	6/12-7/31	Chrysops (4 sps.?) Tabanus (3 sps.?)	824	---	Neg.	0	No record of susceptibility check. No record of susceptibility check.
77	6/3-19	T. venustus	40	---	Neg.	0	No record of susceptibility check.
80	8/17	T. erythraeus	65	---	Neg.	+	
113	8/1-22	T. sulcifrons	86	80	Pos.	+	Died of anaplasmosis.
115	6/5	T. rubescens	85	---	Neg.	+	
117	8/22-25	T. atratus	19	---	Neg.	+	
118	8/25	T. lineola	8	---	Neg.	0	
142	7/13-17	T. benedictus	23	---	Neg.	0	Killed due to poor condition.
168	8/2-23	T. erythraeus	140	---	Neg.	--	Failed to show anaplasmosis bodies after blood inocluation.
169	6/15-8/4	Chrysops sp. T. atratus T. erythraeus	47	---	Neg.	+	

Table III.—Immediate and Deferred Transfer Feedings from Acute Cases.

Number of cow	Dates of Feedings	Species of Fly Used	Total Number of Bites	Number of Direct Bites	Number of Deferred Bites	Time Deferred in Days	Days from First Bite to Appearance of Anaplasmosis Bodies	Results	Susceptibility of Animal	Remarks
81	6/12-22	Chrysops sp?	41	24	17	1-2	---	Neg.	+	
* 45	6/15-17	T. abactor, sulcifrons, fuscicostatus, venustus, Silvius pollinosa	43	25	18	1-6	38	Pos.	+	Died of anaplasmosis
* 64	6/16-23	T. abactor	79	61	18	1-4	35	Pos.	+	Used as anaplasmosis carrier
* 65	6/15-17	T. sulcifrons	24	16	8	1-2	66	Pos	+	Flies very difficult to feed
73	6/26-7/21	T. fuscicostatus	40	36	4	1	---	Neg.	+	
77	6/3-9	T. fuscicostatus	44	40	4	1	---	Neg.	0	
109	6/30-7/14	T. equalis	85	49	36	1-9	35	Pos.	+	Mild attack. Recovered.
110	7/7-27	Silvius pollinosa	9	8	1	1	---	Neg.	0	Very difficult to get these flies to feed.
111	6/8-10	Silvius pollinosa	73	72	1	1	---	Neg.	0	Very difficult to get these flies to feed. Killed due to poor condition.
119	9/8-10	T. sulcifrons	112	110	2	1	38	Pos.	+	Died of anaplasmosis.
171	6/17-19	T. erythraeus	101	100	1	1	36	Pos.	+	Recovered.
172	6/17-19	T. equalis	155	154	1	1	38	Pos.	+	Later used as carrier.

* Reported by Sanborn et al. 1932.

Table IV.—Immediate and Deferred Transfer Feedings from Carrier Cases.

Number of cow	Dates of Feedings	Species of Fly Used	Total Number of Bites	Number of Direct Bites	Number of Deferred Bites	Time Deferred in Days	Days from First Bite to Appearance of Anaplasmosis Bodies	Results	Susceptibility of Animal	Remarks
* 21	8/19-25	T. abactor, sulcifrons and C. sequax	41	38	3	1-3	108	Pos.	+	Died of anaplasmosis
* 22	8/17-22	T. lineola, sulcifrons and atratus	34	31	3	1-4	---	Neg.	+	
* 67	7/2-28	T. venustus	115	47	68	1-11	61	Pos.	+	Later used as carrier
158	7/20-8/4	T. erythraeus	130	113	17	1-8	---	Neg.	?	Checked by blood inoculation
161	9/18-29	T. erythraeus	252	127	125	1-9	---	Neg.	---	Blood smears and temperature reaction—Negative

* Reported by Sanborn et al. 1930, 1932.

Silvius pollinosa was used twice with no success, the first time with 8 direct and 1 deferred bite, the second time on a different animal with 72 direct and 1 deferred bite. This fly is very difficult to feed and is not a satisfactory experimental insect.

An experiment using a mixed sample of 43 flies belonging to the following species, *T. abactor, T. sulcifrons, T. fuscicostatus* Hine, *T. venustus* and *Silvius pollinosa,* resulted in a clinical case of anaplasmosis. Twenty-five bites were direct and 18 were delayed from 1 to 6 days.

T. sulcifrons was used in two positive transmissions, the first involving 16 direct and 8 delayed bites, showed up 66 days after the initial feed. In the second case, which showed marginal bodies 38 days after the first bite, 110 direct and 2 deferred bites were used.

T. fuscicostatus failed to produce the disease twice, despite 36 direct and 4 deferred bites on one animal and 40 and 4, respectively, on a second. The susceptibility of one animal was proved, that of the second animal was not checked.

Two successful transmissions were obtained by using *T. equalis*. One cow received 49 direct and 36 deferred bites, the second 154 direct and 1 deferred bites. Thirty-five and 38 days, respectively, elapsed before the marginal bodies appeared.

Tabanus abactor and *T. erythraeus* were responsible for the transmission of a single case each, the former by means of 61 direct and 18 deferred bites, the latter by 100 direct and 1 deferred.

Flies Fed on Carrier Animals

In this series of experiments five animals were bitten by flies which had obtained their infection from carrier cows. Delayed feedings in some cases occurred 11 days after the infective feeding. Two of these cows developed anaplasmosis.

Animal No. 21 received 38 bites from a mixed sample of *T. abactor, T. sulcifrons* and *Chrysops sequax* August 19. One, two and three days later it received a single bite from flies which had fed the first day. One hundred and eight days later this animal showed marginal bodies in the blood.

T. venustus was able to transmit the disease from a carrier to a healthy cow by 47 direct bites and 68 bites deferred 1 to 11 days. Sixty-one days elapsed before the appearance of marginal bodies in the erythrocytes.

Three animals failed to sicken after the bites of 34, 130 and 252 feedings, respectively. It should be noted, however, that only one of these animals was susceptible as the other two failed to react when inoculated with virulent anaplasmosis blood.

(3) DEFERRED FEEDINGS ONLY

Since the time a fly remains infective is a very important factor in the spread of disease, eight experiments were set up with the hope of determining this factor. Periods of deferment ranged from 5 minutes to 21 days, and both acute and carrier cases were used to infect the flies.

Despite the use of large numbers of flies and feedings extended over a long period, this series was entirely negative. Tables V and VI summarize these experiments.

DISCUSSION

As stated in the introduction, the purpose of these experiments was to determine, as far as possible, the role of horseflies in the spread of anaplasmosis.

It is unfortunate that small laboratory animals cannot be used in experimental work, as the cost of obtaining and maintaining cattle prohibits the use of numbers large enough to be statistically valuable. However, it is believed that the 49 cases reported here indicate the role of the horsefly even though conditions were unnatural and the numbers were relatively small.

The first series of experiments represents an attempt to demonstrate the ability of tabanids of several species to carry anaplasmosis directly from a sick or carrier animal to a normal one. This is the condition most likely to occur in a herd containing such an animal.

When flies begin to feed on an animal, the normal reaction is to dislodge them by whatever means is available: switching the tail, shaking the head, stepping, or walking through brush. It is obvious that when animals are close together and a fly is disturbed in its feeding, the chances that it will return to the same host to finish its feeding are not much greater than that it will attack a second animal. Therefore, the immediate transfer of flies interrupted in feeding seems to be a logical procedure in experimental work on transmission.

Table V.—Deferred Feedings Only From Acute Cases.

Number of cow	Dates of Feedings	Species of Fly Used	Total Number of Bites	Number of Direct Bites	Number of Deferred Bites	Time Deferred in Days	Days from First Bite to Appearance of Anaplasmosis Bodies	Results	Susceptibility of Animal	Remarks
62	8/17-9/5	T. abactor	136	---	136	1-15	---	Neg.	+	
173	6/20-30	T. equalis	26	---	26	1	---	Neg.	+	
175	6/23-7/3	T. erythraeus	92	---	92	1-2	---	Neg.	+	
231	6/29-8/2	T. abactor	85	---	85	5 min.	---	Neg.	+	
233	7/1-30	T. abactor	78	---	78	1-5	---	Neg.	+	
234	7/13-8/2	T. abactor	46	---	46	1 hr.	---	Neg.	+	

Table VI.—Deferred Feedings Only From Carrier Cases.

Number of cow	Dates of Feedings	Species of Fly Used	Total Number of Bites	Number of Direct Bites	Number of Deferred Bites	Time deferred in days	Days from First Bite to Appearance of Anaplasmosis Bodies	Results	Susceptibility of Animal	Remarks
66	6/15-7/6	Chrysops sp?	225	---	225	1-21	---	Neg.	0	
94	4/22	T. lasiophthalmus	2	---	2	1	---	Neg.	0	Killed due to poor condition.
	4/24	T. oklahomensis	2	---	2	1	---	Neg.	0	
240	8/2-14	T. abactor	100	---	100	1	---	Neg.	+	Checked by blood inoculation.

An analysis of the immediate transfer feeding data shows that, with one exception, all susceptible animals that received more than 12 bites from flies previously feeding on an animal with a clinical case of anaplasmosis developed anaplasmosis as a result. The one exception, No. 229, was bitten 53 times over a period of 34 days; consequently, it was exposed to a small number of bites daily.

Flies which had previously fed only on a carrier animal were not efficient vectors, however, as only one positive case, animal No. 113, was developed from 11 experiments involving from 19 to 824 flies. Unfortunately, the animal which received the largest number of bites, No. 74, was not shown to be susceptible and No. 168 was definitely non-susceptible. However, cow No. 115, a susceptible animal, was exposed to only one less bite than No. 113, yet developed anaplasmosis from 86 *T. sulcifrons* feedings. This suggests that carrier animals are not very dangerous to have in a herd unless large numbers of flies are present, because the possibility of a single animal receiving the necessary number of interrupted bites is rather remote. This statement applies only to horseflies, however, and does not include ticks or mechanical transmission.

The addition of deferred bites in the second series of experiments seems to add little if any to the likelihood of infection regardless of the source of the infecting bites. A smaller number of direct transfer bites was not able to carry over the disease when supplemented with many interrupted feedings. Table IV seems to involve a slight contradiction of the foregoing statement, however, as a composite sample of three species of flies transferred anaplasmosis to No. 21 with 38 direct and 3 deferred bites from a carrier. This number is smaller than that required in the direct transfer feedings from carrier animals alone.

The third series of experiments produced only negative results despite the fact that bites were delayed only five minutes in one case and up to 20 days in another.

Tsetse flies are capable of spreading African sleeping sickness and nagana both immediately and after a period of several days, in the first case because of contaminated mouthparts and in the second due to a life cycle in the body of the fly. The second and third series of experiments indicate that horseflies can spread anaplasmosis only by means of contaminated mouthparts and not biologically, as no evidence of a life cycle could be obtained either experimentally or histologically.

Purely mechanical transmission of other diseases by horseflies has been reported by several authors. Mitzmain (*13*) succeeded in transmitting surra from animal to animal by direct transfer of *T. striatus* Fab. Using the same species of fly he was able to transmit anthrax (*14*) 45 seconds to 30 minutes after an infective feed. Herms (*15*) and Nuttall (*16*) record cases of infection of veterinarians with anthrax by the bite of horsefly. Francis et al. (*17*) have shown that tularaemia may be spread from animal to animal or animal to man by means of horseflies.

It seems safe to assume that flies remain infective with anaplasmosis for only a short period of time, less than 5 minutes during the warm summer months, and can be important in the spread of the disease only if an acute or carrier animal is in the same herd or one located a very short distance away.

The number of flies required to convey the infection from a carrier animal to a normal one is much greater than that necessary if the infecting animal is suffering from a clinical case of anaplasmosis. It is possible that this factor may partially explain the small number of cases directly attributed to fly bites. A large enough concentration of horseflies to spread anaplasmosis from carriers cannot be built up until the fly season is fairly well advanced. The incubation period for the development of anaplasmosis is usually sufficiently long to make the clinical cases appear after the fly population has started to decrease. As a result there are seldom enough flies at this time to spread the disease, even from clinical cases. Occasional outbreaks may be explained by the development of clinical cases earlier so that they are present during the height of the fly season. Tick transmission or man-made mechanical transmission may be responsible for the initial clinical cases.

As was previously pointed out, tabanids are probably more able than any other biting arthropod to transmit a number of diseases in a purely mechanical manner. The large size of these flies and the admirable construction of the mouthparts may be responsible for this fact. The biting mechanism of a horsefly is composed of 9 parts, 3 of which are median and unpaired while the other 6 represent 3 pairs of lateral appendages. Medially the most anterior part is the labrum. It is shaped like a dagger but somewhat blunt at its point. Its proximal lateral edges are overlapped by the maxillary palpi, which are two sensory appendages of the maxillae. Posterior and lateral to the labrum are 2 pair of long, slender, tapering blades. The anterior pair are mandibles, the posterior pair maxillae. The mandibles are broad, flat, chitinized blades, slightly recurved on their inner edges. The cutting area is limited to the distal portion of the inner edge and consists of a row of very fine teeth somewhat like those found on a hand saw. These mandibles are moved by powerful adductor and abductor muscles. The biting portion of the maxilla is a stout, chitinous rod, quadrilateral in section at its distal part but flattened proximally. The entire surface of the distal end and approximately one-third of the mesial surface is armed with stout, rasp-like teeth.

The second median structure, the hypopharynx, is posterior to the mandibles and between the maxillae. It resembles the labrum in shape but is much thinner and more slender and comes to a sharper point so that the tip is soft and flexible. This structure contains the duct of the salivary gland and conveys its fluid into the wound.

When the mouthparts are in their normal position, all of these structures except the maxillary palpi are surrounded by the labium. It is a soft organ having the distal third divided into two labella. These are bilaterially symmetrical oval lobes covered internally by a membrane bearing a series of minute canals which enable the fly to absorb fluids from moist surfaces.

Biting is initiated when the fly settles on the skin of the host, selects with the aid of tactile hairs a suitable place for feeding, and retracts the labella to expose the mandibles and maxillae. These structures function as a saw and file respectively and, by rapidly repeated movements, cut a hole in the skin. When the blood level is reached, the labella are applied to the surface. The blood (prevented from coagulating by the salivary secretion) runs along the grooves on the inner surface until it reaches the food canal and is sucked into the body.

It is obvious that the mouthparts, of necessity, become contaminated with the blood of a host and that the blood will remain in a fluid condition for some time because of the salivary secretion. If this fly bites a second animal a short time after feeding on an anaplasmosis carrier, it is indeed difficult to see how contamination of the wound with blood from the previous host can be prevented.

Furthermore, it seems evident that blood from an animal in the clinical stage of the disease is more likely to cause an infection in the second animal than is blood taken from a carrier animal. The much larger number of anaplasma bodies and the lower viscosity of the blood from a clinical case would seem to make this necessary.

In the light of these experiments, of the epidemiological evidence gathered, of the very apparent structural adaptation of horseflies to the mechanical transmission of pathogenic organisms, and of the proven ability of these flies to mechanically transmit surra, anthrax, and tularaemia, it seems necessary to assume that horseflies may play an important part in the spread of anaplasmosis during the warm, dry season in the mid-continent region of the United States.

SUMMARY AND CONCLUSIONS

1. Epidemiological evidence suggests that horseflies may play an important part in the transmission of anaplasmosis.

2. A sufficient number of bites by horseflies which have just fed on clinical or carrier cases of anaplasmosis will transfer the disease to healthy animals, as evidenced by 15 out of 49 positive transmission experiments.

3. Fewer bites are required if the infecting animal is in the acute stage of the disease than if it is a carrier.

4. Bites obtained five or more minutes after the infective feed were not able to transfer anaplasmosis to healthy animals.

5. No evidence of biological transmission by horseflies was obtained.

6. Anaplasmosis has been transmitted by seven species of *Tabanus* and there is little reason to believe that any species of this genus cannot spread the disease if it is able to draw blood.

7. Horseflies may be important vectors of anaplasmosis among animals in the same herd but can have little influence on the spread of the disease to animals more than a short distance away.

8. The mouthparts of *Tabanus* are well adapted to spreading blood diseases such as anaplasmosis.

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