I. COLLECTED STUDIES ON THE INSECT TRANSMISSION OF TRYPANOSOMA EVANSI.

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1. THE RELATION OF TABANUS STRIATUS TO SURRA DISSEMINATION.

Certain species of the *Tabanidae* have been associated with animal trypanosome transmission in the literature whenever the question of an invertebrate host has been considered. These flies have been connected with the direct method of conveyance in several experimental investigations and the latter will be reviewed as follows:

Rogers (1901) experimenting with surra in India used horseflies (species not given) which were caught and kept for varying periods of time after being allowed to bite and suck the blood of an animal which was suffering from surra, and whose blood at the time contained the *Trypanosoma evansi* in large numbers. They were subsequently allowed to bite healthy dogs and rabbits.

In every case in which the flies had been kept from one to four or more days after biting the infected animals, no disease ensued. When, however, flies which had just sucked infected blood were immediately allowed to bite another healthy animal positive results were obtained after an incubation corresponding with that of the disease produced when a minimum dose of infected blood is inoculated into an animal of the same species. The result was uncertain if only one or two flies were allowed to bite and especially if they were allowed to suck as much blood as they wished without being disturbed. If on the other hand several flies which just sucked an infected animal were induced to bite a healthy one and especially if they were disturbed and allowed to bite again several times infection was always readily produced in both rabbits and dogs.

In an experiment described as an example of the method employed, Rogers succeeded in transferring the disease from dog to dog through the biting of 12 horseflies.

Fraser and Symonds (1908) in a study of surra transmission in the Federated Malay States were able to transfer the infection from horses and bulls to the horse, dog, and rabbit in the mechanical method of biting. A single fly was found to be able to convey the disease. The species used in their experiments were *Tabanus minimus, T. partitus, T. vagus*, and *T. fumifer.*

Flies infected 24 hours produced surra by inoculation into susceptible animals, but all attempts from 1 to 10 days proved negative.

Ed. and Et. Sergent (1905) (1906) in two papers discuss their experiments in the transmission of nagana, dourine, and zousfana, trypanosomiases encountered in Algeria.

They succeeded in transmitting all three diseases by means of tabanids, *Atylotus tomentus* and *A. nemoralis*. They investigated especially the dromedary trypanosomiases, el-debab. and zousfana and concluded that tabanids could transmit these diseases by immediately successive bites. In their field observations they noticed that when several dromedaries were neighbors the flies bite first one and then another without interval of repose, precipitating themselves upon the animals in a swarm but rarely succeed in sucking blood at the first attempt until a fatigued or less attentive animal no longer repulses them—"So many successive bites so many lancet stabs." The experimental data presented demonstrated that it was possible to convey the trypanosomes from mouse to mouse with a single tabanid at a single thrust of its lancets when there was no interval between the bites, but when intervals of 15 minutes to 70 minutes were permitted to elapse infection by "stabbing" could not be carried. The Sergents also demonstrated mechanical transmission from rat to rat and from rat to mouse by means of the biting of two to eight flies. Also that six to eight tabanids could convey infection through their bites at an interval of 22 hours. After 24 hours no infection resulted and the flies did not harbor trypanosomes in their intestinal tracts.

Bruce and his collaborators (1910) working with *Tabanus secedens*, *T. fuscomarginatus*, and *T. thoracinus* and an African cattle trypanosomiasis (*Trypanosoma pecorum*) found that these tabanids appeared to be unable experimentally to transmit trypanosomes by the mechanical method of transmission. They could not state whether the flies could convey the disease after a period of development of the trypanosome. Direct application upon monkeys of flies from small cages proved unsatisfactory; therefore, in the hope that *Tabanidae* would live longer and feed more readily, a fly-proof kraal was constructed in the natural haunts of the fly.

In this experiment a calf was exposed to the bites of flies in a compartment shared by the infected calf. A healthy control calf was placed in another compartment. The wild flies used did not live more than three to four days in spite of the presence of running water, shrubs and foliage in the fly compartment. The experiment was declared negative.

Leese (1911) conducted a series of experiments with *Tabanidae* to demonstrate the rôle of biting diptera in the transmission of surra under natural conditions in India. He succeeded in conveying

Trypanosoma evansi with flies (*Tabanus* and *Haematopota*) removed from surra-infected camels in the open to a guinea pig and a white rat. Ten specimens of *Haematopota* and four specimens of two species of *Tabanus* were used in the direct mechanical method.

In another paper, Leese (1912), reviewing his experiments incriminating *Tabanidae*, concludes that in the case of surra the mechanical theory of transmission remains a perfectly satisfactory one, and that the theory of a life cycle of development for *Tr. evansi* in an invertebrate host is not tenable except by analogy.

Baldrey (1911), in an experimental investigation of the rôle of tabanids and other flies in surra conveyance, attempted to account for trypanosome development by feeding infected flies upon healthy guinea pigs and horses for 18 consecutive days. No infection resulted either in the direct method or otherwise. Dissected flies within 24 hours after infection when injected into a guinea pig and a pony produced the disease with fatal results. The author shows that *Tabanus* flies feed only once in five days, so that the only method of direct infection would be where a fly is disturbed in feeding and immediately attacks another animal.

Strong circumstantial evidence incriminating tabanids in surra dissemination is given by Mohler and Thompson (1909) in a study of the disease found in an importation of Indian cattle. They found that only 3 cases among the infected cattle originated in India and 15 additional cases developed after landing on Simonsons Island off New York. Three species of *Tabanus* and one of *Stomoxys* were the only flies found on the island. The authors held *Tabanus striatus* responsible for the outbreak.

In a series of experiments aimed to determine to what extent *Tabanus striatus* Fabricus, the common house fly of the Philippines, was involved as a mechanical carrier of trypanosomes the following conclusions were advanced. (P. 229, Phil. Jour. Science, Sec. B., vol. 8, No. 3, 1913.)

1. *Tabanus striatus* Fabricus for the first time recorded has been found to play a rôle in the transmission of surra. Bred horseflies have been employed for the first time in such experiments. Errors resulting from naturally infected wild flies have thus been eliminated.

2. Three experiments were successful in the direct or mechanical transmission by "interrupted" feeding when only a short interval was allowed between the bites on infected and healthy animals. In 16 experiments the minimum number of flies with which the infection could be transmitted was 2.

3. The maximum length of time that *Trypanosoma evansi* has been demonstrated microscopically in the gut of this species of fly after feeding on infected blood is 30 hours; the organisms were found in the fly's dejecta two and one-half hours after biting the infected animals;

and suspensions of flies, when injected subcutaneously, were found infective for animals for a period of 10 hours after the flies had fed on infected blood.

4. Trypanosomes of surra were not found to be transmitted hereditarily in *Tabanus striatus* Fabricus.

One monkey and two horses became infected as the result of the bites of horseflies used in this series. In analyzing the reason for such a small percentage of positive results (3 out of 16 attempts) with the use of animals whose blood was swarming with trypanosomes at the time of fly biting it was concluded that the feeding methods employed restricted the normal behavior of the parasites. In every instance the flies were induced to complete the infective meal upon the healthy host after a single insertion of its proboscis into the sick animal. The feeding is differently conducted by this fly in nature, as it was observed in every instance that a fly required several insertions of its proboscis at short intervals upon different hosts, and was never permitted by the host to become satiated at a single application of the skin-piercing apparatus.

The experiments in the purely mechanical method were therefore renewed with attempts to simulate more natural behavior of the blood-sucking gadfly. The two experiments bearing on this principle resulted successfully, both animals, a horse and a bull, showing undisputed evidence of the disease. The latter evidently recovered from the infection and later proved immune to inoculation of large doses of blood containing numerous trypanosomes which produced fatal results in a monkey. The horse proved infectious after an incubation period of five days, showing a moderate number of trypanosomes then and many times previous to its death which occurred on the fifty-second day after the bites were received.

TO DETERMINE IF A SINGLE FLY CAN TRANSMIT THE DISEASE.

In previous experiments nine trials with single flies resulted negatively. Here the fly was permitted to bite infected and healthy animals once only. Obviously not sufficient numbers of organisms were transferred on the probe of the fly to cause infection in this artificial way. In the present experiment a tabanid was induced to feed with the maximum number of interruptions upon two hosts alternately. Successive applications from a glass tube were made on surra infected horse and healthy horse. The interval during the transfer was only a few seconds, not more than 20 in any instance. In this manner each animal was bitten at short intervals a total of 26 times during 35 minutes by the same fly, which, however, did not appear abnormally engorged when removed for observation. The healthy horse, which was isolated in a screened stall, passed through an incubation period of nine days, when a few typical trypanosomes

were found in its blood. The animal died 67 days after the fly biting was performed. Heart's blood which contained numerous trypanosomes was injected into a monkey. The latter died upon the eleventh day with trypanosomes swarming in blood taken from its ear.

An additional trial with a single fly was made with monkeys as blood donors. Here an interval of 15 minutes was permitted between bites on infected and healthy hosts. The fly made three distinct stabs with its proboscis into the skin of both monkeys and could not be induced to continue.

Six days after the biting the healthy monkey showed a febrile condition and a moderate number of organisms in its circulation. Fifteen days later the animal died with swarms of *Trypanosoma evansi* in blood removed from his heart.

THE QUESTION OF THE LENGTH OF TIME TABANUS STRIATUS REMAINS INFECTIVE.

In all of the experiments in the direct mechanical method previously reported no provision was made for an interval longer than 1 minute. Consequently the limit of infection in these flies was not ascertained. This problem was taken up in the present investigation in an attempt to imitate as far as practicable the natural procedure of fly biting in the interrupted method. From the data presented in the table following, it is learned that 15 minutes is the maximum time that *Tabanas striatus* is able to infect through its biting.

TABLE I.—*Experiments in the direct method of transmission.*

Interval after biting infected host.	Trypanosome count of infected host when bitten.	Number of flies applied.	Total number of bites.	Healthy animal exposed.	Fate of animals exposed to fly bites.
3 minutes.....	Swarming...	7	35	Horse 135 ...	Positive on 9th day; monkey inoculated; died in 8 days; horse 135, died in 42 days. Disease also reproduced in bull No. 38.
5 minutes.....	Scanty to swarming.	26	133	Horse 344....	Positive; 10 days incubation. Test monkey contracted surra, dying in 11 days; horse 344, died in 60 days.
Do.....	Swarming...	2	6	Monkey X..	Negative; succumbed later to blood inoculation.
7–8 minutes.....	Numerous ..	30	97	Horse 123....	Positive; 6 days incubation; dead in 38 days. Reproduced surra with fatalities in monkey and guinea pig.
10 minutes.....	.....do.....	12	16	Horse 120....	Positive; 5½ days incubation; dead in 35 days. Its blood killed 1 monkey and 2 guinea pigs.
15 minutes.....	Moderate to swarming.	18	18	Horse 300...	Negative; tested later and found susceptible to surra.
Do.....	Swarming...	1	3	Monkey 13..	Positive; died with numerous trypanosomes in heart in 21 days.
20 minutes.....	Scanty to swarming	30	102	Bull 38.....	Negative; inoculated with blood of infected horse 30 days later. Showed trypanosomes and recovered.
1 hour.....	Swarming...	12	12	Horse 339....	Negative; 1 fly after feeding injected into a guinea pig produced surra.
2–3 hours.....	Numerous...	10	10	Monkey 14..	Negative.
3–4 hours.....	Swarming...	39	39	Horse.....	Do.

EXPERIMENTS IN THE INDIRECT METHOD OF INFECTION.

Many attempts were made to keep alive large numbers of gadflies under artificial conditions in order to test the biological application of development to the experimental transmission of *Trypanosoma evansi*. This was finally achieved with a limited number of flies to the extent of 26 days in two experiments and 21 days in one experiment.

Methods.—In this series the flies were kept, when not applied on animals, in a large locally constructed refrigerator where the temperature was quite uniformly maintained at 23° to 25° C. Here flies were kept in a large breeding flask generously supplied with dry filter paper laid lengthwise in roughly rolled strips. When it was desired to feed the flies they were transferred to individual wide-mouthed test tubes plugged with cotton wool.

The parasites, after the initial engorgement on the surra-infected host, were applied in the various experiments once daily, although the individual fly bit on an average only once in three days. When the flies became disabled they were injected as soon as practicable in saline suspensions into guinea pigs. The following table presents a summary of the data in transmission experiments in the indirect method:

TABLE 2.—*Experiments in the indirect method of transmission.*

Interval after biting infected host.	Number of flies applied.	Healthy animal exposed.	Trypanosome count of infected host when bitten.	Fate of animals exposed to fly bites.
1–2 days	33	Horse 339	Swarming	Negative.
1–2 days	15	Horse 73	do	Do.
3–10 dyas	59	Horse 339	do	Do.
3–10 days	105	Horse 73	do	Do.
3–10 days	28	Horse B	Numerous	Do.
11–14 days	41	Horse 339	Swarming	Do.
11–14 days	87	Horse 73	do	Do.
11–14 days	25	Horse B	Numerous	Do.
15–19 days	15	Horse 339	Swarming	Do.
15–19 days	22	Horse 73	do	Do.
15–19 days	8	Horse B	Numerous	Do.
21 days	10	Horse 339	Swarming	Do.
21 days	13	Horse 73	do	Do.
21 days	2	Horse B	Numerous	Do.
23 days	6	Horse 339	Swarming	Do.
24–26 days	7	Horse 73	do	Do.
24–26 days	4	Horse 339	do	Do.

Results of experiments in the indirect method were strengthened by a number of instances of inoculations of flies into susceptible animals at various intervals after biting the infected host. Dissections and microscopical examinations for trypansomes were made prior to injecting.

Flies disabled by injuries or age were ground up in physiological salt solution and injected subcutaneously into guinea pigs and in one instance into a horse. Flies were inoculated after the following periods of possible infectivity:

Into guinea pigs: 2–3 days, 6 days, 9 days, 12 days, 14–15 days, 16 days, 17 days, 19 days, 21 days, 24 days.

Into a horse: 25 days and 26 days.

No suspicious protozoan organisms were encountered in the flies dissected and apparently no developmental forms were present as the animals inoculated remained normal one month following the experiments.

EVIDENCE OF INFECTION CONVEYED IN THE PROBOSCIS.

It often occurs in an outbreak that where large numbers of horses are corraled that only one animal of the lot may contract surra when it is obvious that the others are exposed to the same agencies. In two instances of this sort the writer collected tabanids, immediately after segregation of the sick horse, from animals which escaped infection and found in dissections in a few instances numerous surra-like organisms. A practical problem thus presented itself. How many horses could flies infect successively? Experimentally this would resolve itself into the determining if an infected fly in biting cleans its contaminated proboscis at a single insertion, or if infective organisms are retained so that when interrupted in the biting process infection may be carried to the next animal bitten. Or, in other words, is the conveyance of infection effected through the stab of the fly or assisted by the physiological action of regurgitation or activity of the salivary glands? To determine this point an experiment with horses and tabanids was conducted as follows: Three horses screened from each other were exposed to the successive bites of flies which had contaminated their labiums with blood of a horse whose blood was swarming with trypanosomes at the time of the experiment. A small trapdoor communicated between the screened stalls, permitting of the transfer of glass tube and fly. An attendant was stationed in each stall, so that the feeding could be better facilitated. Each fly was fed 30 seconds on the infected horse, and without interval 30 seconds on horse No. 58, followed immediately by 30 seconds on horse B, and after an interval of 5 minutes from the time of the infective bite, the fly was permitted to complete the meal on horse No. 73. In this manner 43 flies fed on the sick horse, 43 on the first contact horse No. 58, 39 flies bit the second contact, horse B, and 32 bites were completed upon horse No. 73.

After an incubation period of six days horse No. 58 showed a temperature of 40.3° C., followed by a morning temperature of 41.2° C. At this time blood from an ear vein revealed a moderate number of quite typical surra organisms. Sixty-two days following the fly applications horse No. 58 was down and struggling in the final stages of the disease. Numerous trypanosomes were seen in blood removed from the ear. One cubic centimeter of blood removed

from the jugular vein at this time was injected into monkey No. 75, which died with typical indications of surra infection 11 days later.

Horses Nos. 73 and B were held under observation for 40 days following the experiment, during which time no reacton was noted. Blood inoculated into monkeys and guinea pigs proved negative for trypanosomes.

It is concluded from this experiment that the biting of *Tabanus striatus* is innocuous in infecting more than one horse as a result of a previous contamination.

STUDY OF AN OUTBREAK OF SURRA IN WHICH TABANUS STRIATUS WAS THE PROBABLE AGENT OF INFECTION.

The writer was fortunate enough to have an opportunity to make an epidemological study of an outbreak of surra where intimate relations between host and carrier could be identified. This occurred from May to July, 1913, on the Government stock farm, 26 kilometers south of Manila, where a breeding station was maintained. Here the mares and colts were pastured while the stallions were quartered in darkened stables adjoining the pastures. The pastures for the brood mares were interspersed with groves of trees which afforded shade for the animals and incidentally very convenient resting places for the thousands of gad flies observed during the outbreak. During the heat of the day when tabanids and other flies were least active the herd was distributed among four stables which are designated in the table below as approximate centers for the dissemination of new cases of surra. During the hours of 10 a. m. to 4 p. m. the horses were pastured on land contiguous to the respective stables, the grazing areas being partitioned by wire fences and natural barriers of groves of trees. The outbreak of surra occurred when gad flies were the most prevalent day biting parasites. These flies originated from the shores of an 80-mile lake which was located 640 meters from the stock farm. A natural connection between lake and the reservation was afforded by an unbroken row of rain trees which attracted many hundred of resting flies at all times.

The source of the present outbreak is supposed to have originated in a mare which was brought to the stock farm for breeding the latter part of April, 1913. The disease in this animal was not recognized as surra, although it died with suspicious symptoms. The infection was identified May 9 in horse No. A–86, which was removed to the research laboratory for treatment of surra. This was followed one day later by three cases found in grazing animals stabled 400 meters from horse No. A–86. Three days later a horse kept in a stable 1,060 meters distant showed pronounced symptoms. From the last stable a total of 13 animals were found infected. In the nearest stable, a distance of 1,060 meters, seven cases developed. Two hundred and ten meters

from this stable two horses were removed and two more cases were found in the next stable about 200 meters distant from the preceding. It was observed that by far the greatest area (about 80 per cent) of woodland and stream was included in the tract from which the greatest number of cases was removed. Here the total number of horses present was about the same as that in the next pasture. Without doubt the greatest number of gad flies was observed and collected from the region where much water and woods abounded.

Systematic blood examinations of the horses were not made until the first five cases were identified, i. e., after the infection gained a foothold, so that it is not possible to state accurately the incubation period of the disease in the cases detected.

In collecting horseflies from the focus of infection several specimens of *Tabanus striatus* were removed from trees and also from horses showing engorged abdomens with swarms of trypanosomes. Surra was reproduced in a monkey and three guinea pigs by injections of suspensions of material of this sort.

The table following gives a list of cases of the infection with their respective dates of detection and a statement of the distances from the previous cases the animals were removed. These distances are approximate only, using as a basis the distance of one stable from another relative to the location of sick horses.

TABLE 3.—*Relation of cases to place of infection.*

No. of horse.	Date of detection.	Approximate distance from last case.	No. of horse.	Date of detection.	Approximate distance from last case.
		Meters.			*Meters.*
A-86	May 9, 1913		99	May 28, 1913	1,180
B-99	May 10, 1913		7	May 29, 1913	1,380
215	do	210	45	June 2, 1913	1,380
270	do		264	June 3, 1913	1,060
113	May 12, 1913	1,060	B-112	June 9, 1913	1,060
B-17	May 20, 1913	1,060	B-87	do	1,060
206	May 21, 1913	1,060	108 and B-53	June 13, 1913	Nil.
291	May 27, 1913	Nil.	179 and B-71	do	Nil.
182	do	210	A-9 and 115	June 16, 1913	Nil.
B-65	May 28, 1913	1,180	2	June 26, 1913	1,380
B-44	do	1,180			

Not any of the stallions which were quartered in darkened stables contracted the disease. Daily examinations of these quarters showed an absence of gadflies although the stable inhabiting species, *Hippobosca maculata* and *Stomoxys calcitrans* in addition to several species of mosquitoes were encountered.

In reference to the epidemiological study of the surra outbreak on the Government stock farm no evidence was presented to support any possible biological development of the surra organisms relative to an invertebrate host. Indeed the evidence is against the development of any latent infection. The horses and cattle under surveillance

were examined by microscope once weekly after the last case of horse surra was isolated. No clinical symptoms of the disease were manifested during four months following the blood examinations.

This study was further advanced by systematically collecting flies from this focus beginning two days following the last case of surra. These flies were applied during a period of two months to two healthy monkeys and the animals examined from time to time for indications of surra. One hundred and four flies were treated in this way without results. These monkeys reacted in the usual manner to injections of small does of virulent blood obtained from a horse which was spontaneously infected during the stock-farm outbreak.

THE OCCURRENCE OF SURRA INFECTION AND THE PREVALENCE OF TABANUS STRIATUS.

The locating of the breeding places of the gadfly, *Tabanus striatus*, occupied the writer's time during two years in studies of the epidemiology of surra in southern Luzon. This region has been notorious for all the time during American occupation on account of its annual loss due to epizootic surra. The disease has always been associated with the proximity of Bay Lake (Laguna de Bay) which gives rise to the Pasig River, at the mouth of which Manila is situated. The lake extends south of Manila for approximately 80 miles along the Provinces of Rizal and Laguna. It serves as an important waterway for native commerce between Manila and lake ports, communicating with interior barrios by means of carabaos and pack ponies. The latter relation is of interest epidemiologically, as the draft animals coming for many miles serve as ready vehicles for the flies which abound along the lake shore.

In a study of the Government archives embracing the American veterinary reports, it was ascertained that a few clearly demonstrated outbreaks of surra have occurred throughout the lake region. The military authorities have reported the necessity for abandoning certain strategic points as cavalry posts due to the tremendous loss of horses from surra. In 1902, as one example there were 113 cavalry horses destroyed in one outbreak of the disease at Santa Cruz and Pillar, towns on the upper end of the lake. Less severe outbreaks have occurred since in this region among officer's mounts.

The veterinary division of the insular government reports that there was a general widespread epizootic in 1908–1910, throughout Laguna Province, involving the loss of hundreds of mules, horses, and carabaos in some 12 towns on the lake shore. In 1909, 20 valuable horses of the Government stock farm at Alabang, Rizal Province, contracted the disease at a time when *Tabanus striatus* was markedly prevalent. In 1913, outbreaks of surra coming under the writer's personal notice occurred at several points along the lake

region and also in Manila. At the latter point enzootic conditions have prevailed from the time of American occupation to the present. The occurrence of epizootic surra in the lake Provinces in the latter part of 1913 has been of unusual interest on account of the coincidental faunal relations, which had been studied for several months prior to the cases observed. An accurate check was afforded to the incidence of surra on the strength of an exhaustive survey of faunal conditions pursued during April to September of the same year. This survey in antedating and correlating the epizootics is considered of tremendous value in supporting results of laboratory experiments. The biological relations were established before there arose the necessity for epidemiological studies. These consisted in a survey of the entire lake shore from a motor boat by water and in a walking tour by land. In all the regions where it was possible to traverse the sandy lake shore the breeding places of *Tabanus striatus* were located. In the detour of the infected region larvæ and pupæ of *Tabanus striatus* were found in abundance on the lake front embracing the following municipalities: Taguig, Balagbag, Alabang, Muntinlupa, San Pedro, Tunisan, Binan, Santa Rosa, Calamba, Los Banos, Bay, Santa Cruz, Jola Jola, Pelilla, Binangonan, and Morong. Flies from these localities were distributed to interior points by the following means: The adult flies were transported on the backs of slow-moving carabaos, bulls, and pack ponies; larvæ and pupæ were often transported in loads of shell and sand for road building and other purposes from the lake to interior points; and flies as they emerged from their habitats on the lake shore if no convenient host were available flew to the nearest rain tree and rested. In many lake towns there was afforded an unbroken communication from lake to the interior of the town by rows of rain trees. These served as the favorite native shade tree throughout these Provinces and were observed to be the most attractive resting place for thousands of both sexes of *Tabanus striatus*.

At every place visited native officials furnished stories of the devastation due to surra infection. Many of these were confirmed by official records and observed cases. Outbreaks were personally investigated at Los Banos, Calamba, Santa Rosa, Alabang, and Manila. In every instance sufficient evidence was obtained to incriminate *Tabanus striatus* as the carrier responsible for the dissemination of *Trypanosoma evansi*.

DARKENED STABLES RELATIVE TO INCIDENCE OF TABANUS STRIATUS.

Many investigators have observed that tabanids do not infest animals kept in a darkened stable. This is assumed by some as significant epidemiologically and others eliminate *Tabanidæ* on the

same grounds. For instance, Schat (1903) claims that cattle and horses in surra outbreaks in Java were not protected against surra when kept in stables because *Stomoxys calcitrans* and *Lyperosia exigua*, two stables inhabiting flies, had access to them. The horse-fly, *Tabanus* sp. of Java, is stated to play an insignificant part since it does not get inside the stables.

According to Laveran and Mesnil (1907) during the Mauritius surra epizootic the cattle belonging to the Indians were noticed to be immune on account of being boxed up in small, dark huts into which the the flies did not penetrate.

The influence of dark stables in India was commented on by Leese (1911), who states that much can be done in lessening the number of flies without interfering with the supply of fresh air by extending the eaves of stables as far downward on all sides as the height of the pony will admit. "I was not able to get the disease to spread in my controls by the few flies which penterated into the stable until they were tied up immediately outside of the protection afforded by the low roof. Only two or three *Tabanus* were seen biting in our stable during the whole surra season, although they were often seen just outside it."

Evidence of the protection against surra infection in the faunal conditions maintained in darkened stables is offered in the following example:

Horse No. 256 had been kept in an open corral and used for the saddle during November and December, at the end of which month it was placed in a partially darkened shed in company with other stock. On January 10, 1913, the attendant, noticing an unusual condition in the horse, reported the fact to the veterinary inspector, who found a temperature of 40.1° C. and ordered the removal of the animal. The native attendant neglected to isolate the animal until January 18, when an examination of its blood revealed numerous trypanosomes. Then the horse was removed to the screened stable and a careful examination made of the horse's companions. During the six weeks which followed not any of the 11 horses and 4 bulls showed symptoms of disease.

While the sick animal was quartered in the open shed many stable flies and hippoboscides were seen, but gadflies were not in evidence. One specimen of the latter species had been collected from the lighted portion of this shed 18 months before, but there were no recurrences, although the work bulls entered the shed with as many as 30 to 40 tabanids infesting them. These flies were seen to leave their hosts upon entering the interior of the shed.

Additional evidence of the protection afforded by darkened stables is presented in a practical experiment conducted in Manila during the time of maximum tabanid infestation. In this trial 3 horses and 2

carabaos were placed in a partially darkened shed, where 3 surra-infected carabaos in contiguous stalls were exposed to biting flies. The eight animals were not disturbed for a period of 2 months, during which time the infesting flies were collected and recorded.

Culicoides, Phlebotomus, and various species of mosquitoes were observed in addition to stable flies and *Lyperosia*.

During the entire period two specimens of *Tabanus striatus* were collected. The latter species was collected from carabaos in the pasture adjoining to the extent of 60 to 70 in a few minutes.

After exposure to the fly-biting experiment the animals were segregated in screened stalls and observed for 30 days with the usual precautions. No infection was found in the five healthy animals and the experiment was judged to be negative.

REFERENCES TO LITERATURE ON TABANIDAE.

ROGERS. The transmission of the *Trypanosoma evansi* by horseflies, and other experiments pointing to the probable identity of surra of India and Nagana or Tsetse-fly disease of Africa. Proc. Roy. Soc. (1901), vol. 48, pp. 163–170.

FRASER and SYMONDS. Surra, Studies from the Institute for Medical Research, Federated Malay States (1908), No. 9.

ED and ET. SERGENT. Etudes sur les trypanosomiases de berberie en 1905, Ann. Inst. Past. (1906), vol. 20, p. 665.

ED and ET. SERGENT. El-Debab, Trypanosomiase des dromadaires de l'Afrique du Nord. Ann. Inst. Past. (1905), vol. 19, p. 17.

BRUCE and others. Experiments to ascertain if Tabanidae act as the carrier of *Trypanosoma pecorum*. Proc. Roy. Soc. (1910), vol. 83, No. B–565, p. 349–357.

LEESE. Experiments regarding the natural transmission of surra carried out at Mohand in 1908. Indian Civil Veterinary Department Memoirs (1911), No. 2.

———. Biting flies and surra. The Journal of Tropical Veterinary Science (1912), vol. 7, No. 1.

BALDREY. The evolution of *Tr. evansi* through the fly: *Tabanus* and *Stomoxys*. Journal of Tropical Veterinary Science (1911), vol. 6, No. 3, p. 271–282.

MOHLER and THOMPSON. A study of surra found in an importation of cattle followed by prompt eradication. Twenty-sixth Ann. Rep. Bur. Animal Ind. (1909), 81–98.

2. THE RELATION OF MOSQUITOES TO SURRA.

Mosquitoes have been suspected of disseminating surra in the Philippines since the earliest investigations. However, equal stress has been placed on the incrimination of all of the blood-sucking forms. Concerning the relation of mosquitoes, the native people of the Philippines believe strongly in the guilt of these insects as trypanosome transmitters. Many believe that protection can be afforded domestic animals by grazing at distances from lowlands which breed mosquitoes.

Working in Manila, Curry (1902) summarizes his studies on surra conveyance by emphasizing stable flies rather than mosquitoes, which latter, he states, Howard wrote him should be looked upon with suspicion.

Leese (1911) considered that he had satisfactorily eliminated *Anopheles* and *Culex* and *Stegomyia* in several surra transmission experiments performed in India. His experiments gave no support to the factor of a developmental cycle of *Tr. evansi* in the invertebrate host. His conclusions are based on experiments at intervals after infective biting of 2 days with 58 mosquitoes to 11 to 16 days with 4 mosquitoes.

Dutton, Todd, and Hannington (1907) failed to transmit *Tr. gambiense* in their experiments with Anopheline mosquitoes *Pyretophorus costalis*. Dissections were made 12 hours to 11 days after infection. The insects were kept alive by feeding every two days on uninfected animals. *Trypanosoma gambiense* was seen in periods ranging from 12 to 42 hours following the infective meal. They quote some authors who show that *Tr. gambiense* survives ingestion by mosquitoes for only 36 hours, and remains virulent to mice for a period of 14 hours.

The possibility of the transmission of trypanosomes by mosquitoes has been shown by Roubaud and Lafont (1914) in an investigation conducted in French West Africa. *Stegomyia fasciata* was used in experiments with *Trypanosome gambiense* in rodents and monkeys. Only the direct method of transmission was attempted. Mosquitoes in these experiments harbored viable trypanosomes for more than 24 hours.

In a large series of experiments with surra in the Philippines I was not able to transmit the infection with several species of mosquitoes with animals kept in screened stables, in screened cages, or by applying possibly infected mosquitoes from glass tubes and bottles upon many healthy animals.

In the experiments with monkeys the mosquitoes, after feeding on infected horses were applied from a large bottle, into which the monkey's tail was thrust and held from 1 to 2 hours, until all of the insects had fed. In several experiments the monkey was placed in a small cage after it was denuded of much of its hair and immobilized for 3 hours daily, during which time the mosquitoes were observed to feed.

The character of the infection induced by these insects in the latter experiment would rest almost wholly in transmission by the indirect method or infection after a considerable interval. These nematocerous insects are not involved in interrupted feeding in nature, since the biting to the point of engorgement is completed almost wholly upon the same host. However, in experiments mosquitoes easily adapt themselves to interruption in the feeding process. The mouth is immediately withdrawn when forcibly interrupted and feeding reassumed in an adjoining area upon the skin of the host's body.

The experiments in the direct method of infection are included in the table following. Two species, namely, *Aedes (Stegomyia) calopus* and *Culex fatigans* are considered in relation to the various hosts.

TABLE IV.—*Experiments with mosquitoes.*

AEDES (STEGOMYIA) CALOPUS.

Period during which mosquitoes were transferred.	Infected animal used as donor.	Number of days trypanosomes were present.	Healthy host exposed to mosquito bites.	Number of mosquitoes transferred from sick host seen biting healthy animal.	Interval between feedings.	Date of conclusion of experiment.
May 17–27	Horse 40	5	Horse 44.	134	20 minutes to 40 hours...	June 23
May 11–29	....do	12	Monkey 5	113	30 minutes to 48 hours...	June 15
June 23–29	Horse 42	7	Mule 3...	621	20 minutes to 4 hours....	Aug. 2
Aug. 2–19	Horse C	18	Monkey 7	654	10 minutes to 1 hour.....	Sept. 5
June 5–22	Horses 37 and 42.	14	Horse 43.	884	15 minutes to 1½ hours...	June 22

CULEX FATIGANS.

July 3–12	Horse C	10	Horse 44.	984	15 minutes to 1½ hours...	July 15
July 14–Aug. 20	....do	38	Horse B..	979	5 minutes to 8 days.....	Sept. 1

The matter of hereditary transmission was likewise disposed of in an experimental way in conjunction with the indirect method. Three hundred specimens of *Aedes (Stegomyia) calopus*, collected during the act of biting a horse which had numerous surra trypanosomes in its blood, were placed in a screened stable with a healthy horse. Here the insects were encouraged to lay their eggs in vessels provided for the purpose. The larvæ which developed were removed from time to time and placed in suitable containers in a separate stall where another healthy horse was provided for the mosquitoes emerging.

No mosquitoes remained after 36 days, when the experiment was concluded, and the horses were further observed during 30 days. No infection resulted.

Mosquitoes proved to harbor surra trypanosomes a greater length of time than any other blood-sucking form. Several specimens of *Aedes* (*Stegomyia*) *calopus* were found with active trypanosomes resembling *evansi*, for a period of 42 hours after an infective bite. These were located in the proventriculus. None was encountered in the salivary glands or in dissections of the mouth parts in various periods up to 36 days after biting sick animals. In this series 30 hours was the longest period the trypanosomes in these mosquitoes (*Aedes* (*Stegomyia*) *calopus*, *Culex fatigans* and *C. ludlowi*) were found virulent by inoculation into a monkey. The species infected the maximum period was found to be *Aedes* (*Stegomyia*) *calopus*.

MOSQUITO EXPERIMENTATION IN THE FIELD.

The question of the possibility of dissemination of surra through mosquitoes was put to a practical test during an outbreak of the disease at the Government experiment station, 26 kilometers south of Manila. At this location, from May to July, 1913, a total of 24 horses contracted surra. During this period the region was under personal surveillance, in which a careful epidemilogical study was conducted. It was concluded that the Philippine horsefly, *Tabanus striatus* was responsible for the distribution of the infection. The prevalence of this fly was marked a few weeks preceding the first cases diagnosed and a short time following the last cases isolated. The field experiments with mosquitoes were performed during the occurrence of the spontaneous cases toward the end of the outbreak.

Four healthy ponies were placed in an improvised corral immediately adjoining the pasture where the majority of the surra cases occurred. To make the test more severe a pony suffering from the disease was quartered in the same corral and permitted to mingle with the four healthy ponies. The exposure to nocturnal parasites continued during eight nights, when systematic collections were made, by the aid of a hunter's lamp, of the blood-sucking forms molesting the five ponies. Similar collections were made from a few of the horses in the adjoining pasture. Two species of *Anopheles*, one species of *Aedes*, and four species of *Culex* were recognized. Mosquitoes were the predominant forms present; a species of sand fly, *Phlebotomus*, was recognized and found to be quite abundant.

The occurrence of *Tabanus striatus* in the pastures was carefully noted during this test. Their activities were timed to be within the limits of 5 a. m. and 7 p. m. Therefore it was necessary to avoid these hours in order to eliminate the horsefly as a transmitting agent. To be sure, a few diurnal mosquitoes (principally *Aedes* (*Stegomyia*)

calopus) infested horses in this locality, but with the experimental evidence at hand these can be safely ignored for the present.

The five ponies were removed from their screened stalls as immediately after 7 o'clock as was practicable and placed in the open corral until 4.45 a. m. The animals were kept in individual fly-screened stalls during daylight.

After the eighth night the animals were no longer exposed but kept under observation for a long period in the screened stable. A fresh animal was then placed in the corral, where it was exposed during 35 nights in order to attract mosquitoes in an attempt to provide for the possibility of trypanosome infection carried either hereditarily or through a developmental cycle in the mosquito.

After the experiments the five healthy ponies were not removed from the screened stable for a period of 42 days during which time their temperatures were observed and blood examinations made. No reaction was noted, so the experiment was declared negative.

REFERENCES ON MOSQUITOES AND TRYPANOSOMIASES.

CURRY. A report on surra with studies on the mode of transmission. American Medicine (1902), vol. 4, 95–99.

LEESE. Experiments regarding the natural transmission of surra carried out at Mohand in 1908. Indian Civil Veterinary Memoirs (1911), No. 2.

DUTTON, TODD, and HARRINGTON. Trypanosome transmission experiments. Ann. Trop. Med. and Parasit. (1907), vol. 1, 201–229.

ROUBAUD and LAFONT. Experiments on the transmission of human Trypanosomes of Africa by means of the *Stegomyia fasciata*. Bull. de le Soc. de Path. Exotique (1914), No. 1. Borrowed from translation in The Amer. Jour. of Trop. Dis. and Prev. Med. (1914), vol. 1, No. 9658.

3. NOTES ON THE BIONOMICS OF LYPEROSIA EXIGUA AND THE RELATION OF THIS FLY TO EXPERIMENTAL TRYPANOSOMIASIS.

In relation to draft animals in the Philippine Islands, *Lyperosia exigua* de Meij is the most predominant of all species of blood-sucking flies. It is prevalent at all seasons and at all hours; found at night usually at rest on bovines, accompanying mosquitoes and sand flies. On horses this fly is relatively an accidental parasite, preferring the hosts upon whose excreta it lays its eggs; carabao and cattle are selected in this regard.

The egg stage.—Under artificial conditions the captive fly begins egg laying within a few minutes. They are laid on the glass or in a suitable medium either singly or in clusters; when the latter is the case the eggs number 10 usually, but two clusters of 18 and 25 have been observed. The eggs are quite viscid when laid, adhering to the surface of the vessel by their convex sides. The color of the egg is ordinarily white, sometimes white with brown periphery, yellow, and light brown. At all events, the egg darkens perceptibly after incubation has fairly commenced. The white egg when exposed to the air changes in a few minutes to yellow and rapidly to brown.

The size of the egg is, with little variation, 1.30 millimeters by 0.35 millimeters. It is considerably smaller than the usual muscid type of egg, though it has the same general form. The egg shell is relatively thick and tough, due probably to the fact that the eggs are laid normally on the surface of the manure, and is thus constructed to resist exposure.

Although the *Lyperosia* will deposit eggs in empty glass vessels, repeated trials give convincing evidence that these flies will not oviposit on horse manure placed in glass jars. This manure mixed with corn meal or bran is not more attractive. But when eggs and newly hatched larvæ of this species are placed in jars with moist horse manure, development follows and the young forms reach maturity.

Hatching.—In glass tubes eggs fail to hatch unless moisture is provided; they dry up within a short time. About 10 hours after deposition the egg shows signs of embryonic movement, and 4 or 5 hours later the larva emerges. With the aid of the microscope the first sign of hatching can be discerned through the exochorion, consisting of a squirming action. By a pressure of the anal portion and an alternate sliding of the head end a decided piston-like movement

results. The head capsule presses against the cephalic end of the egg as the processes upon which the anal stigmata are set force the body in that direction. Then a backward glide is effected in order, apparently, to bring into play the two cutting spines of the pharyngeal apophyses. These working alternately against the two margins of the micropyle canal, slit ajar the upper portion through which the young larva effects its escape. The latter movement is extremely rapid; it is virtually a glide through the forced open micropyle canal.

The larva.—In appearance the larva is not unlike other muscid larvæ, but is probably whiter and more delicately marked. When hatched it is a footless maggot, 1.5 millimeters long and rather chunky. The white cuticle is contrasted by the black pharyngeal apophyses which terminate in the pair of acute hatching spines directed caudally, and the black anal stigmata which, in this species, are borne on two minute pedunculated processes.

The young larva is extremely active in procuring its food. Unlike the larva of *Stomoxys calcitrans*, it can not survive overnight without food and dies in a few hours when left in a test tube with a decoction of manure or in salt solution. Development in this species is quite rapid when the optimum food conditions are present. When placed in horse manure, growth is retarded. In nature the eggs are laid on the surface of cow and carabao manure, but the larvæ do not remain on the surface, but seek the liquid portions of the food medium. Three days suffice for full larval development, when the puparium is formed. At this stage it is not quite 7 millimeters in length. Preparatory to forming the pupal case the larva imbibes a great amount of the surrounding moisture. In so doing the head is kept constantly quivering from side to side. Almost imperceptibly there ensues a clearing action of the black contents of the intestinal tract, and soon this appears yellowish in conformity with the external cuticle. The body segments contract from the oval end cephalically, and with the invagination of the head capsule the barrel-formed puparium is completed. The color of this rapidly changes from a yellow to a bright terra cotta. The dimensions of the puparium are rather constantly 4 millimeters by 2 millimeters.

The pupa.—The pupal stage requires at least four days, the fly emerging in four to five days ordinarily. Unlike *Stomoxys, Philæmatomyia*, and other muscid flies, the postpupal stage in this species is a condensed period, the adult fly bursting through the puparium in a few seconds and within three minutes is apparently fully developed. With wings spread it is seen walking to the lighted portion of the jar, and at once assumes flight if permitted to escape.

Life cycle.—The entire life cycle of *Lyperosia exigua* as observed at various times under laboratory conditions is summarized in Table I.

TABLE I.—*Showing the length of time required for the life stages of Lyperosia exigua.*

Date.	Material used.	Egg.	Larva.	Pupa.	Cycle.
		Hours.	*Days.*	*Days.*	*Days.*
Sept. 6, 1911	Carabao manure	18	5	5	11
Sept. 19, 1911	do	14	4	5	10
Feb. 14, 1912	Cow manure	16	4	4	9
Feb. 16, 1912	do	16	4	5	10
July 26, 1912	Guinea-pig manure	16	5	5	11
July 27, 1912	Carabao manure	14	4	5	10
Apr. 11, 1913	Cow manure	16	4	4	9
May 26, 1913	do	12	4	4	9

Average life cycle, 9 to 11 days.

The adult fly is prepared to feed within an hour. Both sexes draw blood. The action of biting is not unlike that of the stable fly, but the *Lyperosia* usually requires shorter and more frequent bites for complete satiation.

The fly in nature, like its relative, the horn fly of the United States, usually feeds in a group, but is never found resting upon the base of the horns. When found feeding on the horse, it is never in a swarm which occurs on carabaos and cattle. The parasitism of the horse occurs usually in individual instances.

This species, unlike *Stomoxys calcitrans*, is difficult to keep for laboratory purposes, rarely surviving beyond ten days when fed daily. Therefore, its use in transmission experiments was limited to the direct method.

TRANSMISSION EXPERIMENTS WITH TRYPANOSOMA EVANSI.

In several epizootics in widely separated regions in the Orient and in Africa, species of *Lyperosia* have been suspected to be the cause of the dissemination of trypanosomiases. Schat (1903) in his dissertation on the study of the spread of surra in Java is quite decided in his incrimination of *Lyperosia exigua* in addition to the stable fly. His observations were almost entirely epidemiological and meager experimental evidence is presented. Leese (1912) in a careful survey of epidemiological conditions on surra in India found *Lyperosia* prevalent in enzootic areas where horseflies and other blood-sucking forms were absent, and suggests the probability of *Lyperosia minuta* acting as a mechanical carrier. In his experiments reported in 1911 the results obtained led him to state that this species of fly plays an insignificant rôle as a carrier. Austen (1909) mentions in a footnote that a medical officer in British Africa reported the occurrence of *Lyperosia* in an important rôle as a transmitter of trypanosomiasis.

Lyperosia exigua is found to infest the draft animals of the Philippines in tremendous numbers at a time of the year when surra is present. In this connection it is often associated with the gadfly *Tabanus striatus* and usually with *Stomoxys calcitrans*, the stable fly. It therefore devolves upon the investigator to determine to what

extent *Lyperosia* may play a rôle in the absence of other blood-sucking flies.

In the experimental data presented three methods of procedure are included:

First. Exposing healthy animals and infected animals to the bites of flies under natural conditions. Here counts were made of flies taken in the act of biting the hosts kept in the open.

Second. The experimental animals were exposed in a screened stable to the biting of a specified number of flies collected from known sources.

Third. Bred flies were applied from test tubes upon infected and healthy animals at stated intervals.

First method.—Experiments under natural conditions were pursued during 1911 from the latter part of April to the latter part of October. At the time of the year selected this species of fly abounds in swarms, being the most prevalent in numbers of the blood-sucking flies. The animals used were carabaos and horses. Surra-infected carabaos were used to supply the infection, they being preferred as the primary host on account of being the most susceptible of the draft animals to *Lyperosia* infestation.

The three experiments were conducted with the animals in the open under well-lighted sheds and kept together as closely as practicable. The flies were collected twice daily and only the number of specimens of *Lyperosia* was recorded; other species of parasitic flies were observed but not recorded. The hosts were examined for blood parasites, in addition the temperatures were recorded and the animals observed for clinical symptoms.

The table following gives a résumé of the three experiments conducted:

TABLE II.—*Compilation of experiments in which healthy horses and sick carabaos were exposed to Lyperosia flies in the open.*

	Period of experiment.		
	Apr. 27–June 8.	Aug. 19–Sept. 8.	Sept. 9–Oct. 24.
Sick carabaos used	1	1	4
Horses exposed to flies	1	1	2
Flies taken from carabaos	1,811	487	5,802
Flies taken from horses	23	138	432
Infected carabaos showed trypanosomes	17	0	22

After each experiment the horses were placed in screened stalls and blood examinations and temperatures taken daily for 30 days. No reactions were noted.

Second method.—The next two experiments were attempted with carabaos in a fly-screened cage and the insects from known sources introduced at stated intervals.

A fly-proof cage with a substantial fly-proof entrance, 7.3 meters by 9.1 meters, with a height of 4.5 meters graduated to 3.6 meters

was so partitioned that two surra-infected carabaos and a healthy carabao could occupy adjoining stalls without bodily contact and still be exposed to the bites of flies placed in the inclosure. In the first trial two surra-infected carabaos, Nos. 3228 and 3252, which were in advanced stages of the disease, were placed on one side of the coarse-screen partition and the healthy carabao, No. 16, was selected to be exposed.

The parasites used were *Lyperosia* flies collected in open sheds from healthy work carabaos. These were transferred twice daily to the caged animals and were placed promiscuously in the screened inclosure. The parasites attached themselves to the new hosts quite readily. In all over 5,000 flies were employed during the course of the experiment lasting about one month, from January 12 to February 13, 1912. The healthy animal, carabao No. 16, was not removed to the adjoining inclosure until two weeks later, February 28, when all of the flies had disappeared.

As shown in Table III following, blood examinations of the three carabaos were made daily. Carabao No. 3228 was positive for trypanosomes upon 11 days and carabao No. 3252 was positive for an equal number of days, and between the two carabaos the disease was present in a fly-communicable form during 18 days of the experiment.

TABLE III.—*Résumé of experiment in which sick carabaos and a healthy carabao were exposed to Lyperosia flies.*

Date.	Number of flies placed in the inclosure.		Trypanosome examination in carabaos.	
	a. m.	p. m.	No. 3228.	No. 3252.
1912				
Jan. 12	11	.....	Positive...	Positive.
Jan. 13	170	110	...do.....	Do.
Jan. 15	122	115	...do.....	Negative.
Jan. 16	183	87	...do.....	Do.
Jan. 17	153	106	Negative..	Positive.
Jan. 19	70	.....	Positive...	Do.
Jan. 22	75	.....	Negative..	Do.
Jan. 23	88	.....	...do.....	Do.
Jan. 24	.....	20	Positive...	Do.
Jan. 25	40	48	Negative..	Do.
Jan. 26	103	230	Positive...	Negative.
Jan. 27	.....	112	Negative..	Do.
Jan. 28	75	40	...do.....	Do.
Jan. 29	290	.....	...do.....	Do.
Jan. 30	.....	120	...do.....	Do.
Jan. 31	102	.....	Positive...	Do.
Feb. 2	.....	93	Negative..	Do.
Feb. 3	127	445	...do.....	Positive.
Feb. 4	.....	339	...do.....	Do.
Feb. 5	593	.....	...do.....	Do.
Feb. 6	432	114	...do.....	Negative.
Feb. 7	131	262	Positive...	Do.
Feb. 8	138	70	...do.....	Do.
Feb. 13	131	.....	...do.....	Do.

Carabao No. 16, the healthy contact, showed no evidence of the disease either by temperature reaction or blood inoculation into guinea pigs when released February 28. Subsequent observation

of this animal during its two months' quarantine convinced the observer that the experiment terminated negatively.

A similar experiment was attempted with two infected carabaos as blood donors but with the normal carabao in an isolated screened inclosure. During the course of one month beginning February 28, 1912, more than 3,000 flies were placed on the sick carabaos and transferred at intervals of five minutes to three hours to the stall occupied by healthy carabao No. 7. Many of these flies were observed to settle on the second host and caught while biting. Three months following the last experiment the blood of carabao No. 7 was devoid of trypanosomes and the two guinea pigs inoculated remained negative.

Third method.—Finally a series of experiments was conducted under more strictly laboratory conditions. The direct method of applying the flies was used in six experiments with no appreciable interval between the bites of flies directly removed from the infected host, and in seven experiments with intervals varying from 5 hours to 10 days. The table below gives a summary of these experiments.

TABLE IV.—*Experiments in mechanical transmission with Lyperosia and Trypanosoma evansi.*

Interval between feedings.	Number of flies fed.	Condition of blood on infected host.	Healthy animal used.
30 seconds	2	Swarming	Horse 339.
20 seconds	2	do	Monkey 27; died Apr. 1. Neg.
5 seconds to 5 minutes	23	do	Monkey 26; died Apr. 3. Neg.
50 seconds average	40	do	Horse 69.
5 seconds to 2 minutes	61	do	Horse 277.
10 seconds to 1 minute	40	Numerous	Monkey 30.
14 to 20 hours	30	Swarming; animal dying	Horse 66.
5 to 7 hours	39	do	Horse B–120.
24 to 48 hours	29	do	Horse 982.
1 to 4 days	28	Numerous	Monkey 28; dead Apr. 4 (peritonitis).
5 to 7 days	14	do	Monkey 29; dead Apr. 3. Neg.
1 to 6 days	51	Moderate	Guinea pig K; reacted subsequently to surra by blood inoculation.
1 to 10 days	59	do	Carabao 9.

All of the healthy animals used in the 13 experiments above have proved negative for surra infection. Monkeys Nos. 26, 27, 28, and 29 either died from injuries, naturally, or were killed for purposes of necropsy. These animals showed at post-mortem no lesions indicative of surra.

The blood examinations were also negative. Guinea pigs inoculated from the animals exposed to the fly bites have proved negative after one month to six weeks of frequent examinations.

Carabao No. 9, which received the bites of 59 flies, was six months later inoculated with a suspension of 30 *Lyperosia* flies removed from a surra-infected horse. The carabao's blood yielded trypanosomes after an incubation period of 12 days. This animal died 8 months later and though trypanosomes were present in several organs its

untimely death was due more probably to the cramped quarters in which it was confined. However, two guinea pigs inoculated with the blood of this carabao reacted with surra infection and died as a result.

An attempt was made to eliminate the possibility of transmission of surra organisms through heredity in the fly. Several thousand of flies were liberated in the screened inclosure with two surra-infected carabaos, and in 12 days or less not any of the flies remained. In the meanwhile a new generation of flies was produced from the accumulated manure of the two animals. The new flies in turn fed on the sick carabaos, laying their eggs on fresh depositions of manure. Daily this manure was carefully transferred to a separate compartment where a healthy donkey was quartered. In due time the second generation of flies made their appearance and infested the new host.

The experiment was continued for 5 weeks, and 10 days later blood of the donkey was inoculated into one monkey and used a month later for an experiment in which it was inoculated with blood from one of the two surra-infected carabaos. The animal responded to the inoculation with a high temperature and a moderate number of typical blood organisms on the seventh day and died 18 days later with quite characteristic post-mortem lesions of surra.

SUMMARY OF EXPERIMENTS.

Experiments in the direct method with *Lyperosia exigua* and *Trypanosoma evansi* gave negative results.

Horses, which were exposed under natural conditions to the biting of flies infesting sick carabaos, did not become infected.

When thousands of flies infested sick and healthy carabaos placed in a large screened inclosure no infection was transferred.

Only negative results were obtained in the interrupted method of feeding flies in 13 experiments with various animals. In six trials there was no appreciable interval between the bites of flies removed from the infected hosts, and in seven experiments intervals occurred varying from 5 hours to 10 days. The greatest number of flies employed was 61.

In one experiment with several thousands of flies the possibility of transmission of *Tr. evansi* hereditarily was eliminated.

REFERENCES CITED.

SCHAT. Verdere mededeelingen over surra. Mededeel Proefstation Oost—Java (1903), vol. 3, No. 44.

LEESE. Biting flies and surra. The Journal of Tropical Veterinary Science (1912), vol. 7, No. 1.

——— Experiments regarding the natural transmission of surra carried out at Mohand in 1908. Indian Civil Veterinary Memoirs (1911), No. 2.

AUSTEN. African blood-sucking flies. British Museum of Natural History, London (1909).

4. MECHANICAL TRANSMISSION EXPERIMENTS WITH PHILÆMATOMYIA CRASSIROSTRIS.

It was aimed to investigate, if practicable, every blood-sucking species of fly of the Philippines which appear in sufficient numbers to warrant experimentation. During certain months of the year, principally April and May, *Philæmatomyia crassirostris* Stein appears to equal in numbers the ubiquitous stable fly. This fact seemed sufficient justification to attempt to use this fly in surra transmission experiments.

This species, which has been previously reported from British India and Africa, is noted for its very peculiar mouth apparatus, which at first glance would appear to belong to a nonblood-sucking form. It is, however, a very efficient blood sucker, as can be observed from its engorged abdomen and the wound it makes on the host's cuticle. It is very much like *Musca domestica* in general appearance, but it has the general habits of the stable fly. It prefers cattle for its infestation and for the egg deposition on the manure of these animals.

Eight experiments were tried with guinea pigs in transferring infection at an interval less than one minute. From 6 to 36 flies were applied, when the infected animal's blood contained trypanosomes from moderate to numerous in numbers. In two trials 36 and 29 flies, respectively, were applied on two successive days.

One experiment at an interval of one hour between the bites was attempted with 18 flies from monkey to monkey, and three experiments with 2, 11, and 19 flies were attempted with a 24-hour interval.

Only one experiment in the direct method of transmission proved successful. In this trial monkeys were used during six successive daily applications. The blood of the surra monkey employed during the six days contained trypanosomes moderate in numbers upon four days and numerous to swarming upon two days. Fresh flies were applied daily in the following numbers: 2, 25, 8, 17, 33, 19, a total of 104 for the experiment. Only laboratory-bred flies were employed in this experiment.

Six days following the last application of flies upon the healthy host the latter suffered with fever and had a moderate number of surra trypanosomes in its blood. Beginning three days later the trypanosomes were found swarming in numbers until death, which occurred 14 days after the onset of symptoms. Blood from the

heart of this monkey when injected subcutaneously reproduced the disease in a guinea pig, which died after three months, and in a monkey, which showed an incubation period of only three days. The latter died ten days later in a greatly debilitated condition. Death occurred during the night and a great number of dead trypanosomes were seen on the following morning in blood from the heart. A moderate number of "round bodies" were found in stained preparations from the spleen of this monkey.

5. EXPERIMENTS WITH HIPPOBOSCA MACULATA IN THE TRANSMISSION OF SURRA.

The genus *Hippobosca* has been generally overlooked by investigators who have attempted to determine the rôle of the important blood-sucking flies in the conveyance of surra and other trypanosomiases. However, species of *Hippobosca* have been suspected upon several occasions of transmitting pathogenic trypanosomes; and one, *H. rufipes*, has been proved experimentally to transmit Tr. theileri, the cause of gallsickness in cattle of South Africa. (Laveran and Mesnil, 1907.)

Two species of this genus occur in the Philippines, namely, *equina* and *maculata*, but only the latter was investigated. *Hippobosca maculata* Leach is found upon all draft animals, though rarely infesting the carabao, in all months of the year. The habits of the fly are in most respects dissimilar to all other species. It is often found to be the only parasite present particularly on horses. It appears to be uninfluenced by light reactions, seeming to attack equally well in darkened stables and in open pastures. The feeding peculiarities of *maculata* makes it unpromising as a direct contaminator, as it prefers to finish its protracted meal upon the same host. Experimentally it is difficult to interrupt its biting and requires several minutes to recommence feeding on a second host when an unusual length of time is required before it becomes satiated.

Due to the difficulty of rearing a large number of flies at one time, since each female deposits but an individual progeny, the feeding experiments were conducted except in one instance with wild flies captured from sources known to be surra free.

Twelve experiments in the direct method of feeding were pursued, as indicated in the following table:

TABLE V.—*Experiments in the direct method of feeding with hippoboscid flies.*

Date.	Infected animal used.	Condition of blood relative to trypanosomes present.	Healthy animal used.	Number of flies applied.	Length of time fed on surra host (average per fly).	Interval during feeding (average per fly).	Time required to complete meal on healthy host (average per fly).
					Min. *sec.*	*Min.* *sec.*	*Min.* *sec.*
1912.							
June 13	Guinea pig 127..	Scanty....	Guinea pig 92...	5	3 36	3 0	7 24
June 14	.....do.....	Numerous	Guinea pig 94...	9	3 6	2 26	9 6
June 15	.....do.....	Swarming	Guinea pig G...	6	2 0	2 5	4 50
June 16	.....do.....	...do.....	Guinea pig I...	6	2 10	3 50	8 0
June 17	.....do.....	...do.....	Guinea pig L...	9	2 12	2 20	4 40
June 17	.....do.....	...do.....	Guinea pig M...	6	2 0	3 30	7 0
June 18	.....do.....	...do.....	Guinea pig O...	5	1 48	1 0	7 24
June 18	.....do.....	...do.....	Guinea pig P...	8	2 0	1 30	7 7
June 18	.....do.....	...do.....	Guinea pig Q...	4	2 0	4 0	4 15
June 19	.....do.....	Numerous	Guinea pig 89...	10	1 40	3 50	9 12
June 19	.....do.....	...do.....	Guinea pig 112...	5	1 12	24	5 24
June 20	Monkey B.....	...do.....	Monkey, M.....	10	4 0	2 30	8 0

NOTE.—The flies used were discarded after each experiment.

The experiments in the direct method of application were augmented by a series of feedings on clean animals after the flies were induced to feed on a surra animal whose blood proved by animal inoculation to be pathogenic. Thirty-three flies were fed three days upon monkey B whose blood was heavily infected with trypanosomes during each day of this period. On the fourth day and for 26 successive days the flies were applied to clean animals. The two surviving flies died on the twenty-seventh day of the experiment. The table following outlines the results of the fly feeding tests:

TABLE VI.—*Experiments representing the indirect method of applying hippoboscid flies.*

Times since last feeding on surra host.	Healthy animal used.	Number of flies applied.	Average length of time each fly fed.
			Minutes.
1 day	Guinea pig 93	19	10
2 days	Monkey C	24	10
3 days	Monkey D	19	11
4 days	Monkey G	17	11
5 days	Monkey H	23	11
6 days	Monkey I	22	11
7 days	Monkey J	21	12
8 days	Monkey K	17	10
9 days	Monkey L	14	13
10 days	Monkey M	13	11
11 days	Monkey N	13	10
12 days	Monkey P	11	13
13 days	Monkey Q	10	10
14 days	Monkey S	7	8
15 days	Monkey 1	6	12
16 days	Monkey 2	6	14
17 days	Monkey 3	5	9
18 days	Monkey 4	5	13
19 days	Monkey 5	5	16
20 days	Monkey 6	3	15
21 days	Monkey 7	3	10
22 days	Monkey 8	3	10
23 days	Monkey 9	2	11
24 days	Monkey 10	2	7
25 days	Guinea pig 50	2	10
26 days	....do	2	7

With two bred flies a final experiment was attempted and no infection resulted. As in the above experiment the flies were fed three consecutive days on a sick monkey then applied once daily to a clean monkey. Thirty-three days after feeding upon the infected animal both flies were dead. Forty days after the experiment it was apparent, by blood examination and temperatures, that all of the animals bitten by the hippoboscid flies were free from infection.

REFERENCE CITED.

LAVERAN and MESNIL. Trypanosomes and Trypanosomiases. Nabarro English Edition. Bailliere, Tindall and Cox, London (1907), p. 351.

6. EXPERIMENTS WITH BLOOD-SUCKING GNATS.

Experiments in direct transmission with *Culicoides judicaudus* Bezzi were conducted with captive flies. Ten experiments with monkeys and guinea pigs proved negative. In one trial from monkey to monkey 326 gnats were used to bite both hosts on two successive days, 200 upon the first day and 126 were applied upon the second day. In all of these experiments the gnats were applied individually from small test tubes. The insects were used once only, then discarded.

In one trial during six days the indirect method was attempted. This likewise proved negative.

In regard to other similar blood-sucking forms one species which is indeed ubiquitous during probably five to six months may be considered here. A *Phlebotomus* was observed upon many occasions to enter the fine meshes of brass screening surrounding the stalls housing experimental animals. Infected and healthy horses kept in these stables were attacked in common and several instances were noted of transfer from surra infected to healthy animals. No instance of infection brought about through this means was observed during three years of experience.

In the field experiments with carabaos and horses, and with horses and horses, ample opportunity was afforded for the transmission due to bites of thousands of *Phlebotomus* flies. Conveyance of the disease in any instance did not result on this account.

7. THE RÔLE OF MUSCA DOMESTICA IN SURRA CONVEYANCE.

The possibility of infection conveyed by the house fly in a passive rôle has long been recognized. In reference to trypanosome transmission, it is presupposed that the method is correlated with a previous skin abrasion affording the channel of entrance for the pathogenic organisms. Consideration of skin abrasions occurring through injury or disease must include the mechanical openings effected by various ectoparasites. The latter include such forms as the ticks, gadflies, and stable flies. These parasites are important factors commensurate to the size of the openings punctured in the host's cuticle and which remain after withdrawing the piercing mouth parts in satisfying the desire for blood. It is obvious that in the presence of muscids with mouths constructed for lapping contaminated products unlimited possibilities for conveyance of surra from host to host present themselves.

The conveyance of trypanosome-infected blood by the house fly in the presence of abrasions produced through sores and wounds was taken into account by early workers in trypanosomiasis of the Philippines.

Musgrave and Clegg and Musgrave and Williamson (1903) considered this as an important factor in the prevalence of surra.

These writers state that they transmitted surra infection to healthy animals through the agency of house flies, and more recently Darling (1911) conveyed *Tr. hippicum* to the mule experimentally by means of house flies. Darling concluded one positive and two negative experiments with house flies fed artificially on the blood of an infected guinea pig. In this investigation a few drops of blood were placed on a glass plate, covered with a jar, and 18, 9, and 6 flies in three experiments fed about 5 minutes, and after an interval of 30 seconds were applied for 5 minutes to the shaved scratched skin of three mules. The positive result was obtained in the case of the mule infested with 18 house flies.

In the present study the investigation was pursued from various angles. The purely mechanical method of contamination was not considered until the more important biological relations were exhausted. Only *Stomoxys calcitrans* was considered as a correlative factor in reference to the house fly as a carrier of infection. In a previous report (1912) I have described the intimate relations existing in the feeding habits of the two species of flies. It was pointed out that the coprophagous flies, and especially the house fly, acted in a sense as secondary passive parasites by lapping to a point

of engorgement the blood brought to the host's epidermis by the probing of the labium of the stable fly. It was observed under natural conditions that an unusual percentage of nonbiting flies were attracted to domestic animals not alone to act their rôle as scavengers, but were found to accompany stable flies and gadflies to avail themselves of the food provided through the wounds made by the latter.

With this peculiar phase of parasitism in mind, experiments were conducted to determine the relationship of *Musca domestica* as a carrier of trypanosomes. It was aimed to prove first that this fly could harbor infective organisms, and was determined satisfactorily by numerous dissections and injections of saline suspensions of abdominal contents of flies fed on the abraded tail of the surra-infected monkey. Two guinea pigs and one monkey inoculated with material of this sort sickened after an average incubation period and died giving indications of the nature of the disease at autopsy. Preparations of the blood of the three animals showed *Trypanosoma evansi* in large numbers.

Attempts were made to simulate the normal relationship of parasitism in *Musca domestica* and *Stomoxys calcitrans* by placing many flies of the two species in a common bottle and permitting them to attack the inclosed tail of a surra-infected monkey. Only laboratory-bred flies were employed. Two hundred and fifty selected house flies were allowed to lap the blood from the tail of an infected monkey, upon which 200 stable flies had previously fed. The monkey's tail was withdrawn from the bottle when the majority of the house flies appeared fairly well engorged. Then 200 *clean Stomoxys* were introduced into the bottle with the fed *Musca* and the tail of a fresh monkey placed within. Here the attempts to imitate natural conditions were quite successful, the house flies applying their mouth parts to punctures made by the stable flies. Similar experiments were conducted with the two species of flies in one trial from monkey to monkey, and three trials, slightly modified, from guinea pig to guinea pig. Large numbers of laboratory-bred flies were used, and the infected blood donors in each instance were positive microscopically.

The five experiments were followed by negative results demonstrating under these conditions that *Musca domestica* does not transport infection through the channel afforded by the wound caused by the skin-piercing apparatus of *Stomoxys calcitrans*.

The possibility of surra infection being carried by the fly's feet was tested also. This was performed in the same manner with both species as in the preceding experiments, but with this difference—the infected *Musca* were introduced from a separate bottle, and by careful manipulation of the wire support attached to the monkey's tail the flies were permitted to alight on the appendage, but were prevented from feeding. Two experiments with monkeys involved the application of

30 stable flies and 50 house flies in one instance, and 200 stable flies and 250 house flies in the other instance.

Guinea pigs were also used in three experiments with fewer flies.

The five experiments failed to demonstrate that the wound made by the labium of the stable fly was a suitable channel for the introduction of trypanosomes carried on the pulvilli of *Musca domestica*.

Finally to serve as controls to these experiments four tests were made to decide the question of the possibility of the punctures caused by the bite of the stable fly serving as sites for the introduction of trypanosomes in virulent blood. In these four experiments the bites of 7, 8, 13, and 20 laboratory-bred *Stomoxys* were followed immediately by the rubbing in of freshly drawn virulent blood conveyed on a plantinum loop.

The guinea pig inoculated subcutaneously with similar material contracted surra. The four guinea pigs used for the fly biting escaped infection. One of the latter reacted six weeks later to an injection of the blood of the original blood donor, which died at this time.

The practical significance of the conveyance of trypanosomes obtained by *Musca domestica* from the site of the probing of the stable fly to exposed wounds was finally investigated. Monkeys and horses were employed in this series. The house flies after apparent engorgement of blood derived from the probes of stable flies were transferred to clean bottles and the abraded surface of the monkey's tail presented for the completion of the meal. The two horses exposed were scarified by scratching the haunch with a sharp scalpel.

In transferring bottles and tubes applied to the infected host the mouth of the vessel was carefully wiped with a cloth saturated with strong alcohol. A few minutes were permitted to elapse before the vessel was then applied to the broken skin of the healthy host. Four of the five experiments attempted resulted in positive transmission. One monkey which escaped infection was later proved to be susceptible to subcutaneous inoculation of virulent blood.

The table following shows how the animals and flies were treated in these experiments:

Hosts employed.		Flies employed.		Fate of healthy hosts exposed to infestation of Musca.
Infected.	Healthy.	Stomoxys.	Musca.	
Monkey B...	Monkey L..	70	22	Positive; incubation period 4 days; died 10 days later. Trypanosomes seen in blood from ear and from heart.
Do.....	Monkey T..	92	48	Negative; died later when injected with 2 c. c. of blood of horse 30.
Monkey C....	Monkey R..	100	29	Positive; incubation period 3½ days; blood from ear reproduced the disease in 2 Guinea pigs. Trypanosomes numerous at death in 11 days.
Do.....	Horse 28....	112	40	Positive; incubation period 5 days. Trypanosomes moderate—disease reproduced by injection of blood into horse 27 and 2 guinea pigs. Horse 28 died in 47 days.
Horse 27.....	Horse 30....	98	32	Positive; incubation period 6 days. Trypanosomes numerous—reproduced diseaseby injection of blood into Monkey T and one guinea pig. Horse 30 died after 35 days.

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REFERENCES CITED.

MUSGRAVE and CLEGG. Trypanosoma and trypanosomiasis with especial reference to surra in the Philippines. Publ. Bur. Gov. Labs. (1903), No. 5, pp. 85–87.

DARLING. Experimental infection of the mule with Trypanosoma hippicum by means of *Musca domestica*. Jour. Exp. Med. (1911) vol. 15, No. 4, pp. 365–366.

MITZMAIN. The rôle of *Stomoxys calcitrans* in the transmission of *Trypanosoma evansi*. Philippine Journal of Science (1912), Sec. B. vol. 7, p. 475.